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Exploring the Medicinal Potential of Gishta (Graviola): Identification of Bioactive Metabolites and Silver Nanoparticle Synthesis through Advanced Analytical Techniques

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KEYWORDS

Antimicrobial, secondary metabolites, analytical techniques.

ABSTRACT:

Plants are known for their medicinal value. Many dreadful ailments are being treated by plant extracts. So many plants were proven medicinal, but still many plants have to be proved. Gishta, a typical variety of Graviola species has been found with an antimicrobial nature. The present study mainly aims at identifying secondary metabolites of chloroform and methanol extracts of Gishta, which are thought to be responsible for medicinal properties by preliminary and GC-MS and preparing and characterizing the silver nanoparticles (Ag-NPs) of Gishta (Graviola) seeds extracts using the relevant and pertinent analytical techniques viz., Uv-Vis spectral analysis and scanning electron microscopic (SEM) techniques.

1. Introduction:

Graviola, a short, erect, and evergreen tree species grows about 5–6 m long, leaves are shiny and dark green. It produces large, green, heart-shaped fruits having white fleshy edible pulp. The fruit measures about 15–20 cm in diameter. The different parts of the plant have been utilized conventionally for treating various ailments (Edwards, S., *et al*., 2000). *Graviola* is usually present in the rainforests of different regions of the Earth. The other names of the plant are thorny custard apple, cherimoya, and Brazilian pawpaw. The fruit is also called differently in various languages like soursop (regions of America), cachiman (épineux), shul-ram-fal, hanuman fal, and mullaatha (India), Harar and yebere lib (Ethiopia) means heart of cow (Blackherbals, 2019).

As traditional folkloric medicine seeds and leaves of Gishta are very effective in treating cancer, various parasitic infections, etc. Seeds are known for their emetic function, seed oil kills lice, floral parts are antispasmodic in nature, and fruit pulp has been used as a diuretic and to treat some other ailments. The raw fruit has astringent properties, and cures intestinal problems, whereas ripened fruits are used as antiscorbutic and anthelmintic. The beverage is used as a medical supplement to treat gastric problems. The bark is effective in curing diarrhoea and dysentery. The medicinal properties of the species are attributed to bio-active compounds acetogenins (Ruppercht, J.K., *et al*., 1982). To our knowledge investigation of Gishta for phytochemical composition, antibacterial, antifungal, anticancer, caspase-3 activity of silver nanoparticles has

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not been done especially on this plant species (Saripalli, Harikrishna & Dixit, Prasanna., 2016). This study mainly focused on Phytochemical and GC-MS studies to assert the antagonistic functions of the plant under study.

2. Materials and Methodology:

2.1 Ghista fruits collection:

Fruits of Ghista (Fig. 1a), collected from the Jimma (7°40'26.01"N, 36°50'8.85"E) Oromo region, Ethiopia, in January 2016 after confirmation and authentification deposited voucher specimen number: AAU/CBS/G/2014-01 in College of Biological Sciences, Addis Ababa University, and supplied by Ato Behailu Etana Disasa of Natural Resource Management, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia.

2.2 Extract Preparation:

Seeds of Gishta were dried in the shade at 28 $\pm 3^0$C temperature under aseptic conditions. Then the dried seed materials were powered and subjected to standardized Soxhlet procedure using chloroform and methanol to extract the sample (Harikrishna Ramaprasad Saripalli., 2004). Thus prepared sample was preserved at 4^0C for further research.

2.2.1 Chemicals and Reagents:

All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard.

2.3 Preliminary Phytochemical Analysis:

The phytochemical contents as reported by Harborne (1973), Trease and Evans (1989), Sofowora (1993), and Debela (2002) are subjectively assessed by a chemical test carried out on the extract. The following tests were conducted in triplicate using various concentrations of prepared test sample.

2.3.1 Identification and Detection of Alkaloids:

Each of the plant extracts was dissolved in chloroform solvent. Resultant solution was extracted with dil. H_2SO_4 or dil. HCl. The acid layer obtained was then separated, analyzed for the presence of any alkaloids.

Mayer's test:

Potassium mercuric iodide solution also called Mayer's reagent was added to the acidic solution and observed cream colored precipitate formation, indicating the presence of alkaloids.

Wagner's Test:

Wagner's reagent (Iodine in potassium iodide) was added to the acidic solution and observed for changes. Reddish brown precipitate was observed indicating the presence of alkaloids.

Hagner's test:

Hagner's reagent: Iodine in potassium iodide was added to the acidic solution and observed brown color precipitation which indicates the presence of alkaloids.

Ammonium reinckate test:

Ammonium reinckate solution was added to the acidic solution and observed for any change. Flocculent pink precipitate was observed indicating the presence of alkaloids.

2.3.2 Identification and Detection of carboxylic acid:

Few milliliters of sodium bicarbonate was added to each of the plant extracts (1ml) and observed for any change. Effervescences observed due to CO_2 liberation indicating the presence of carboxylic acid.

2.3.3 Coumarins Identification and Detection:

The plant solvent extracts were treated with alcoholic sodium hydroxide and observed for any color change. Dark yellow color was observed indicating the presence of coumarins.

2.3.4 Fixed oils Identification & Detection:

For these 2 filter papers were taken, each of the various solvent extracts-2ml each were placed on the center of the filter paper and pressed with the other filter paper and observed for any change. Formation of transparent oily spot was observed indicating the presence of fixed oils. 1ml of each of the various plant solvent extracts were taken and added a few drops of 0.5 N alcoholic potassium hydroxide followed by the addition of phenolphthalein (few drops) and then heated 1 to 2h and observed for any change. Soap formation was observed indicating the presence of fixed oils.

2.3.5 Identification & Detection of Flavonoids:

Each type of plant extract (5ml) was made to dissolve in 1ml of alcohol and then proceeded for the following tests.

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Ferric chloride test:

To each 1ml of the above made alcoholic solution, neutral ferric chloride solution (few drops) was added and observed for any change. Blackish red color formation was observed indicating the presence of flavonoids.

Shimoda's test:

Small ribbon of magnesium, few drops of conc. HCL were added to each 1ml of alcoholic extract and observed for any change. A color change from red to pink was noted indicating the presence of flavonoids.

Zinc-HCL reduction Test:

Small amount of conc. HCL and a little of zinc dust were added to each of the alcoholic extracts and observed for changes. Formation of magenta color was noted indicating the presence of flavonoids.

Lead-acetate test:

To 1ml of each alcoholic plant extract, aqueous basic lead acetate solution (few drops) was added and observed for any change. Reddish brown bulky precipitate was formed indicating the presence of flavonoids.

2.3.6 Identification & Detection of Phenols:

Few ml of neutral ferric chloride solution was added to each of the different solvent extracts dissolved in water/alcohol, observed for any color change. Color change indicated the presence of Phenols.

2.3.7 Identification & Detection of quinones:

To 1ml of each of the different solvent extracts, alcoholic potassium hydroxide solution was added and observed for change in color as quinones produce color ranging from red to blue.

2.3.8 Identification & Detection of resins:

Few drops of acetic anhydride, 1ml of conc. H_2SO_4 were added to each 1ml of plant solvent extracts and observed for any color change, color change ranging from orange to yellow indicates the presence of resins. Simultaneously, 1ml of each of the various solvent extracts was diluted with water and observed. Formation of any bulk black precipitate suggests the presence of resins.

2.3.9 Identification & Detection of Saponins:

20ml of distilled water was taken in a graduated cylinder. To this 1ml of each of the solvent extracts were added individually and agitated for about 15min. Any foam formation is indicative of saponins presence.

2.3.10 Steroids Identification & Detection:

Each of the solvent extracts was dissolved in 5ml of chloroform separately. This is considered as stock solution for testing below.

Salkowski test:

To the above-mentioned stock solution, 1ml of conc. Sulphuric acid was added and rested for 5min. Later mixed, on proper mixing a golden yellow color forms in the bottom indicates steroids presence in the sample.

Liebermann Buchard Test:

Chloroform, a few drops of acetic anhydride and 1ml of conc. H_2SO_4 were added one by one to the side of the test tube and rested for about 5min. Brown ring formation at the junction of the two layers plus green upper layer is indicative of steroid presence.

Noller's Test:

Chloroform, pinch of tin foil, 0.5ml thionyl chloride were added to each 1ml of different solvent extracts and gently heated if necessary, any pink color formation is indicative of steroid presence.

2.3.11 Tannins Identification & Detection:

5ml of each of plant solvent extracts dissolved in little water individually, then filtered. The resultant filtrates were considered as stock to proceed for following testing:

Ferric Chloride test:

Few drops of ferric chloride was added to each of the above-mentioned stocks and observed for any change. Formation of blackish precipitate is indicative of tannin presence.

Gelatin Test:

To the above-mentioned filtrate, gelatin solution was added. White precipitate formation is indicative of tannins presence.

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Lead acetate Test:

Few drops of lead acetate solution (basic) was added to the filtrate. Formation of reddish brown bulky precipitate indicates tannins presence.

2.3.12 Identification & Detection of Xanthoproteins:

1ml of the respective solvent extracts were treated individually with small amount of conc. HNO_3 and NH_3. Development of reddish orange precipitation indicates the occurrence of xanthoproteins.

2.3.13 Identification & Detection of Glycosides:

Each of the plant solvent extracts (5ml) were hydrolyzed with 5ml conc. HCL, boiled for few hours in water bath. The resultant hydrolysate is considered as stock for further testing.

Legals Test:

1ml of pyridine and a few drops of sodium nitroprusside solution were added to 1ml of each hydrolysate (stocks) and turned alkaline with NaOH.

Borntrager's Test:

1ml chloroform was added to each 1ml of hydrolysate the chloroform layer was then separated and to this equal amount of dil. NH_3 solution was added and observed for a color change. Any color change is indicative of glycoside presence.

2.3.14 Identification & Detection of Polyketides:

Add few drops of Liebermann's reagent (acetic anhydride and sulfuric acid mixture) to the test solution and heat gently. After heating, formation of a green color indicates positive test for the presence of polyketides. The intensity of the green color can vary depending on the concentration and nature of the polyketides present in the sample.

2.4 Phytochemical profiling using GC-MS analysis:

For GC/MS analysis, the obtained extract of 1g redissolved in 5ml of DMSO (Dimethyl sulfoxide) and prepared the sample for analytical studies. 2µl of injection volume of methanol extract of the seeds of Graviola was used for GC-MS analysis.

2.4.1 Gas chromatography:

The components of a sample are divided and a spectral signature is generated for the sample by gas chromatography. The sample is put into the gas chromatography device's injection port, where it is vaporized, as part of the operation. Following vaporization, the individual components are then separated and examined, with each ending in a very unique spectral peak. The retention time, which is used to distinguish between the components, is the amount of time that passes between the injection and elution of a sample component from the column. An additional measure that provides information on the concentration and kind of sample components is height (Sermakkani M. and Thangapandian V, 2012).

2.4.2 Mass spectroscopy:

Electrically charging a specimen's molecules allow mass spectrometry to analyze it. Following an acceleration via a magnetic flux, these molecules fracture into charged pieces (Gomathi, D. et al., 2015).

2.4.3 GC-MS Specifications:

An GC MS 5975 C Agilent33 was used to perform the Gas Chromatography-Mass Spectrometry (GCMS) analysis. GC CONDITION: Column Oven Temperature 70˚C, Injector Temperature 240˚C, Injection Mode Split, Split Ratio 10, Flow Control Mode Linear Velocity, Column Flow 1.51 ml/min, Carrier Gas Helium 99.9995% purity, Injection volume 1 ml; Column Oven Temperature Program: Rate: 0-10, Temp: 70 - 300˚C, Hold Time 3 – 9 (35.0mts total); COLUMN VF-5ms – Length 30.0m, Diameter 0.25mm, Film Thickness 0.25um); MS CONDITION MS: Scan range: 40 – 1000 m/z; Ion source temperature: 200°C; interface temperature: 240°C. Ionization Equilibrium (-70 eV), solvent cut time of 5 minutes, MS start time of 5 minutes, and MS finish time of 35 minutes scan speed of 2000 WILEY 8, FAME, MS LIBRARY-NIST 08s, and lastly, at 900C/min to 2500C. With 1µl of the split ratio of 10:1.

2.4.4 Identification of Components:

Using the National Institute of Standards and Technology (NIST11.L) database, which contains more than 62,000 patterns, bioactive chemicals are identified by interpretation of the mass spectrum using GC-MS. Each unidentified compound's mass spectrum was compared to the known compounds' kept in the NIST collection. To identify bioactive chemicals in examined test samples, the name, molecular weight, structure, type, and activity of the compounds were ascertained.

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3. Results:

3.1 Phytochemical screening:

The results of qualitative pharmocognostic assess of solvent extracts of Gishta seeds are shown in Table 1. Phytochemical profile of chloroform extracts of Gishta revealed and highlighted the presence of Alkaloids, Fixed oils, Flavonoids, Phytoketoids, Terpenoids, Quinones, Steroids and Xanthoproteins. Whereas methanolic extracts of Gishta recorded the presences of phytoconstituents like Carboxylic acids, Flavonoids, Phenols, Phytoketoids, Fixed oils, Terpenoids, Alkaloids, Resins, Steroids, Quinones, Tannins, Xanthoproteins, Glycosides and Saponins. These constituents may be accountable for the efficient capping and chelating agent of nanoparticles and responsible for exhibiting biological and bio efficacy activities.

Table No. 1. Phyto chemical constituents identified in Chloroform, Methanolic extracts of Gishta seeds using Qualitative chemical tests.

Phytochemical Constituents	Chloroform Extract	Methanolic Extract	Biological activities (P Tiwari et al. 2011)
Alkaloids	Pr	Pr	Antimicrobial, Anthelmintic, Antidiarrheal
Carboxylic acids	Ab	Pr	Antimicrobial, flavorings
Coumarins	Ab	Ab	Antimicrobial
Fixed oils	Pr	Pr	Antimicrobial, Antidiarrheal
Flavonoids	Pr	Pr	Antimicrobial, Antidiarrhoel
Phenols	Ab	Pr	Antimicrobial, Anthelmintic, Antidiarrhoel
Polyketides	Pr	Pr	Antitumor, anticancer, Antimicrobial
Terpenoids	Pr	Pr	Antimicrobial, Antidiarrhoel
Quinones	Ab	Pr	Antimicrobial
Resins	Ab	Pr	Adhesives, incense, perfume
Saponins	Ab	Pr	Antidiarrhoel
Steroids	Pr	Pr	Anti-inflammatory, Immunosuppressors
Tannins	Ab	Pr	Antimicrobial, Anthelmintic, Antidiarrhoel
Xanthoproteins	Pr	Pr	Precursors of antimicrobials
Glycosides	Ab	Pr	Antidiarrhoel

Pr – Present; Ab- Absent

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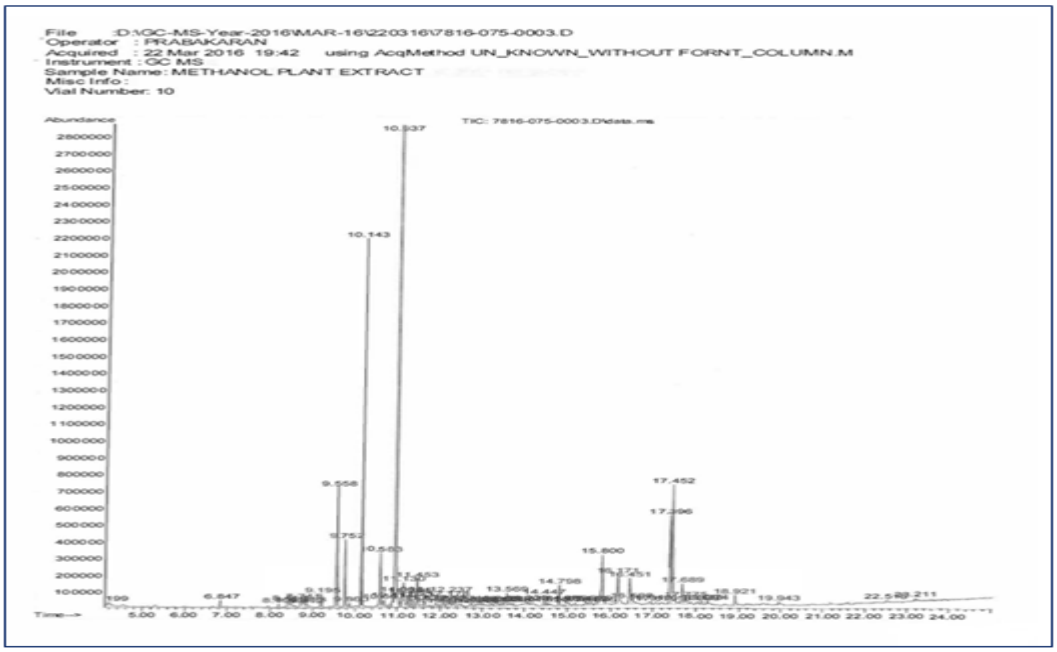

Figure 1: GC-MS Chromatogram of methanol extracts of Gishta seeds

3.2 GC-MS analytical tool:

Gas chromatography-mass spectrometry (GC-MS) is an analytical tool that is used to separate, quantify and analyze organic compounds that are naturally volatile.

3.3 GC-MS Validation – Parameters:

3.3.1 Retention Time:

Retention time refers to the time a compound is retained within the gas tubing. This point is measured from the time that a sample is injected to the time that a sample finally elutes from the column.

3.3.2 Size of the peaks of the spectra:

Peak size is another parameter that is accustomed to present the results of gas chromatography. The greater the scale of the peaks, the upper the concentration of a component of a sample.

Mass-to-charge Ratio (m/z)

The data from the mass spectrometry is utilized to identify this crucial parameter. Applied to the electric charge it carries, this ratio shows the mass of a particular particle. It is known as the m/z ratio. Each component has a limited range. Together with the mass of the complete particle, the fragments' mass spectra are utilized as a puzzle piece. It offers a precise way to identify a chemical and can be conclusive.

3.3.3 Peaks data representation of methanolic extract of Gishta seeds:

The GC - MS chromatogram of the methanolic extract of Graviola seeds exhibited 70 peaks exhibit three best hits indicating the presence of around two hundred and ten compounds (Figure 1). Total GC-MS chromatogram run time was 25 minutes, three types of chemical compounds of each peak with their retention time (RT), molecular weight, the molecular formula was represented in Table 1. The Caryophyllene, Bicyclo [7. 2. 0] undec-4-ene, 4, 11, 11- trimethyl-8-methylene Bicyclo [7. 2. 0] undec-4-ene, 4, 11, 11-trimethyl-8-methylene compounds showed a moderate percentage of peak areas (13.39%) of the chromatogram. Whereas the 9, 15-Octadecadienoic acid, methyl ester, (Z, Z)-9,12-octadecadienoic methyl ester, (E, E)- 9, 12-octadecadienoic acid (Z, Z)-, methyl ester 9-octadecenoic acid (Z)-, methyl ester 9-octadecenoic acid -, methyl ester (Z)- appeared more than the minimum percentage of peak areas (3.30%) of the chromatogram. While the highest percentage of peak areas of chromatograms noticed for the compounds identified as 1H-Cyclopenta [l, 3] cyclopropa [l, 2]

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benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl) -, [3aS-(3a. alpha., 3b. beta., 4. beta., 7. alpha., 7 aS*)]-Bicyclo [4. 4. 0] dec-l-ene, 2-isopropyl-5-methyl-9-methylene-. gamma. -Muurolene (42.53%). In addition to these, a lot of other chemical compounds identified in trace amounts were notified in less than 0.5% peak area counts in the methanol extract of Graviola seeds. A list of all the bioactive compounds is mentioned with respective peak areas in Table.2 corresponding to the GC-MS chromatogram Figure 1. The high peak area analytes of GC-MS chromatograms were ascertained in Table 6 alongside their chemical nature and pharmacological activities.

Table No. 2 : GC-MS analysis of bioactive compounds in methanol seeds extract of Gishta.

Peak	Compound name	% Peak Area	Retention time	Molecular weight g.mol-1	Molecular formula
	3-Piperidinemethanol	0.19	4.198	115.17	$C_6H_{13}NO$
	Sarcosine, N-isobutyryl-, heptyl ester			89.09	$C_3H_7NO_2$
	Butanedioic acid, dimethyl ester			118.09	C4H6O4
	3-Tetradecene, (E) -	0.32	6.850	196.37	C14H28
	3-Tetradecene, (Z) -			196.37	C14H28
	3-Tetradecene, (E) -			196.37	C14H28
	2H-Azepin-2-one, hexahydro-1-methyl 1-Acetamide,	0.18	8.195	127.1842	C7H13NO
	2,2-dichloro-3			339	C12H7Cl4NO2
	3-Dimethylpiperidine			228.33	C12H24N2O2
	DiFluoro-7-hydroxy bicyclo[2.2.l] heptane	0.43	8.410	130.16	C7H11FO
	2,4-Decadienal			152.23	C10H16O
	(E,E)-3-Heptyne, 5-methyl			138.25	C10H18
	Benzeneethanamine,	0.35	8.485	121.18	C8H11N
	N-methyl-Acetamide,			73.09	C3H7NO
	2-chloro-Metaraminol				
	2,4-Decadienal, (E, E)-	0.67	8.373	152.23	C10H16O
	2,4-Decadienal			152.23	C10H16O
	2,4-Decadienal, (E, E)-			152.23	C10H16O
	Amphetamine	0.12	8.812	135.21	C9H13N
	1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide			265.35	C14H23N3O2
	Amphetamine			135.21	C9H13N
	Benzeneethanamine,	0.26	8.856	135.21	C9H13N
	N-methyl(-)-Norephedrine			151.21	C9H13NO
	Cathine			151.21	C9H13NO

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	.alpha.-Cubebene	0.66	9.198	204.35	C15H24
	.alpha.-Cubebene			204.35	C15H24
	.alpha.-Copaene			204.35	C15H24
	.alpha.-Copaene	4.34	9.555	204.35	C15H24
	Copaene			204.35	C15H24
	.alpha.-Cubebene			204.35	C15H24
	1H-Cyclopenta[l,3] cyclopropa[l, 2]benzene, octahydro-7-methyl-3-methyl alpha., 3b. beta., 4. beta.,/ alpha.,/ aS*)] -	2.51	9.755		
	1H-Cyclopenta[l,3] cyclopropa[l, 2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl) -, [3aS-(3a. alpha.,3b.beta.,4.beta.,7.alpha.,7 aS*)]-				
	.beta.-copaene			204.35	C15H24
	N-Methylallylamine	0.19	9.859	71.12	C4H9N
	Undecane			156.31	C11H24
	p-Hydroxyamphetamine			151.21	C9H13NO
	Caryophyllene	13.39	10.141	204.35	C15H24
	Bicyclo [7.2.0] undec-4-ene, 4,11,11- trimethyl-8-methylene			204.35	C15H24
	Bicyclo [7.2.0] undec-4-ene, 4,11,11 -trimethyl-8-methylene-			204.35	C15H24
	Humulene	2.13	10.580	204.35	C15H24
	Humulene			204.35	C15H24
	4-Terpinenyl acetate			196.29	C12H20O2
	2-Pyridinemethanamine, . alpha. -methyl-	0.48	10.669		
	Phenylephrine			167.2	C9H13NO2
	Benzenepropanoic acid, alpha.-(1-aminoethyl)-, [R-(R*,R*)]-				
	1,6-Cyclodecadiene, l-methyl-5-methylene-8-(1-methylethyl) -, [S-(E, E)]-	0.33	10.877	136.23	C10H16
	Bicyclo (4.4.0) dec-1-ene, 2- isopropyl-5-methyl-9-methylene-			204.35	C15H24
	.gamma.-Muurolene			204.35	C15H24
	1H-Cyclopenta [l, 3] cyclopropa [l, 2] benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl) -, [3aS-(3a. alpha.,3b. beta.,4. beta.,7. alpha., 7 aS*)]-	42.53	10.936		

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	Bicyclo [4. 4. 0] dec-l-ene, 2-isoprop yl-5-methyl-9-methylene-				
	.gamma.-Muurolene			204.35	C15H24
	Tetratetracontane	0.59	11.085	619.2	C44H90
	Sulfurous acid, pentadecyl 2-propy1 ester			82.08	H2O3S
	Hexadecane			226.44	C16H34
	.gamma.-Elemene	1.32	11.129	204.35	C15H24
	.gamma.-Elemene			204.35	C15H24
	Santolina triene			136.23	C10H16
	.alpha.-Farnesene	0.62	11.241	204.35	C15H24
	lH-Benzocycloheptene, 2,4a,5,6,7,8 ,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis) -				
	.alpha.-Farnesene			204.35	C15H24
	Phenol, 2,4-bis(l,1-dimethylethyl)	0.53	11.293	362.5	C24H26O3
	Phenol, 2,5-bis(l,1-dimethylethyl)			278.4	C18H30O2
	Phenol, 2,4-bis(l,1-dimethylethyl)			362.5	C24H26O3
	1,5 Cyclodecadiene, 1 methylene-8-[1-methylethyl-S-E, E)	0.40	11.343	136.23	C10H16
	Naphthalene, 1,2,4a,5,6,8 a-hexahydro-4, 7-dimethyl-L -{ l-methylethyl) -			128.170	C10H8
	.gamma.-Muurolene			204.35	C15H24
	Naphthalene, 1,2,3,5,6,8a- hexahydro-4,7 -dimethyl-1-1-(1-methylethyl-,(1s-cis)-	1.30	11.456		
	Benzoic acid, 4- ethoxy-, ethyl ester				
	Naphthalene, 1,2,3,5,6,8a- hexahydro-4,7 -dimethyl-1-1-(1-methylethyl-,(1s-cis)-				
	Sulfurous acid, butyl tetradecyl ester	0.33	11.627		
	Sulfurous acid, butyl tetradecyl ester				
	Decane, 2,4,6-trimethyl				
	Al -gly, 4trlmethylsilyl ester	0.26	11.895		
	Methyl.pent-4-enylamine				
	Benzeneethanamine, N-methyl			121.18	C8H11N
	(5S)-5-Methyl-3-[(2R,8R,13R)-2,8,13-trihydroxy-13-{(2R,5R)-5-[(1R)-1-hydroxytridecyl]oxolan-2-yl}tridecyl]furan-2(5H)-one	0.14	11.984	594.6	$C_{35}H_{64}O_7$
	Benzenemethanol •alpha -(1-aminoethyl) -, (R*,S• J -			44.08	C2H6N

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	(.+/-.)				
	,4-Benzenedicarboxamide N, N '-bis-2-hydroxy - 1 - methyl - 2 -phenylethyl)			164.16	C24H20N2O4
	(-) -Norephedrine				
	1,3-Adamantanediacetamide	0.32	12.080	250.34	C14H22N2O2
	Pterin-6-carboxylic acid			207.15	C7H5N5O3
	N-Acetyl 9-ethoxyamphetamine				
	Columbine	0.53	12.177	358.4	C20H22O6
	2,4-Bis (hyd::"oxylamino)-5-nitropyrt emidine				
	E-14-Hexadecenal			238.41	C16H30O
	5-Eicosene{E)	0.57	12.236	280.5	C20H40
	9-Nonadecene			266.5	C19H38
	N-Acetyl -2 -methylampl□etami no				
	Eicosane	0.38	12.325	282.5	C20H42
	Ethylamine, 2-(adamantan- 1 -y l) 1-methyl-			45.08	C2H7N
	Sulfurous acid, butyl undecyl ester			82.08	H2O3S
	1·-(3,5 -Dimethyl-1 -adamantanoyl)semicarbazide	0.28	12.734	265.35	C14H23N3O2
	Imidodicarbonic diamide, N -formyl-			103.08	C2H5N3O2
	Acetamide, 2, 2 -dichloro			59.07	C2H5NO
	Benzeneethanamine, 4-methoxy-.alpha.-methyl-	0.22	12.845	121.18	C8H11N
	Amidephrine			244.31	C10H16N2O3S
	Ethylamine , 2- (adamantan-1-y l)- l -m			45.08	C2H7N
	Acetic acid, Hydroxyl (1-oxo-2-propenyl) amino]-	0.28	12.897	60.05	C2H4O2
	Benzeneethanamine, 2,5-dimethoxy- alpha.,4-dimethyl-			121.18	C8H11N
	dl-Alanyl-dl-norleucine			202.25	C9H18N2O3
	l-Methyl-2-phenoxyethylamine	0.19	13.001		
	p-Hydroxyamphetamine			151.21	C9H13NO
	5-Nitro-3-cyano-2(1H)-pyridone			165.11	C6H3N3O3
	3,6,9,12-Tetraazatetradecane-l,14-diamine	0.21	13.180		
	N-Methyl-2-phenyl-l-propylamine				
	1-(3,5-Dimethyl-l-adamantanoyl)semicarbazide			179.22	C9H13N3O
	Methanesulfonamide, N,N-dimethyl	0.31	13.321	95.12	CH5NO2S
	Methanesulfonamide, N,N-dimethyl			95.12	CH5NO2S
	Tetratetracontane	0.15	13.432	619.2	C44H90

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	Phenethylamine, p, .alpha.-dimethyl				
	Benzenemethanol, . alpha. -(1-aminoethyl)-, (R*,S*)-(.+/-.)-			108.14	C7H8O
	2-methyltetracosane	0.21	13.470	352.7	C25H52
	10-Methylnonadecane			282.5	C20H42
	Heneicosane			296.6	C21H44
	2-Butenediamide, 2-methyl-, (Z)-	0.10	13.521	114.1	C4H6N2O2
	4-Fluorohistamine				
	2-Amino-1-(o-hydroxyphenyl)propane			151.21	C9H13NO
	Hexadecane	0.57	13.566	226.44	C16H34
	2-methyloctacosane			408.8	C29H60
	Octacosane			394.8	C28H58
	Propanamide, 2-methyl-	0.13	13.729	73.09	C3H7NO
	3-Chloropropionamide			107.54	C3H6ClNO
	Benzeneethanamine, 4-methoxy-.alph a.-methyl-			121.18	C8H11N
	Tetradecane	0.16	14.034	198.39	C14H30
	Sulfurous acid, butyl tridecyl ester			82.08	H2O3S
	Decane, 2,3,5-trimethyl-			142.28	C10H22
	S(-)-Cathinone, N-acetyl	0.10	14.071	191.23	C11H13NO2
	Methanone, (4-methylphenyl) phenyl			29.027	CH2O
	Methanone, (4-methylphenyl) phenyl			29.027	CH2O
	Trichloroacetic acid, pentadecyl ester	0.44	14.450	163.38	C2HCl3O2
	Trichloroacetic acid, pentadecyl ester			163.38	C2HCl3O2
	1-Nonadecene			266.5	C19H38
	3,3-Dimethyl-4-methylamino-butan-2 -one	0.16	14.524	129.199	C7H15NO
	N-Methyl-2-phenyl-1-propylamine			149.23	C10H15N
	N-acetyl-3-fluoroamphetamine			195.23	C11H14FNO
	Isopropyl myristate	0.16		270.5	C17H34O2
	Isopropyl myristate			270.5	C17H34O2
	Isopropyl myristate			270.5	C17H34O2
	Arginine	0.26	14.963	174.2	C6H14N4O2
	,1, 6-dimethyl-2 {propan-2-yl)-l, 3, 5 -dithiazinane				
	2,3-Dimcthoxyamphetamine				
	Hentriaccntane	0.19	15.022		

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	Nonadecane			268.5	C19H40
	Octadecane, 1-chloro-			288.9	C18H37Cl
	Imidazole-5-carboxylic acid, 2-amino-	0.16	15.171	127.1	C4H5N3O2
	Benzenethanamine, N-methyl			253.4	C18H23N
	Di (pent-4-cnylJanine				
	Piperazine, 2-methyl-	0.16	15.416	100.16	C5H12N2
	Benzeneethanamine, 4-methoxy-.alpha.-methyl-			121.18	C8H11N
	5-Isoxazolepropanamlne, N-methyl-3 -(4-nitrophenyl)-				
	2H-Azepin-2-one, hexahydro-1-methyl-	0.16	15.460	127.18	C7H13NO
	N-Methylallylamlne				
	1, 3-Propanediamine, N-rr.ethy1			102.18	C5H14N2
	2H-Azepin-2-one, hexahydro-1-methyl-	0.24	15.542	140.18	C7H12N2O
	Pentane, 2,3,3-trimethyl.			258.44	C16H34O2
	Mexiletine			179.26	C11H17NO
	2H-Azepin-2-one, hexahydro-7-methyl-	0.17	15.639		
	2H-Azepin-2-one, hexahydro-7-methyl-				
	2H-Azepin-2-one, hexahydro-7-methyl-				
	Hexadecanoic acid, methyl ester	2.73		270.5	C17H34O2
	Pentadecanoic acid, 14-methyl-, methyl ester				
	Hexadecanoic acid, methyl ester			270.5	C17H34O2
	Dibutyl phthalate	1.69	16.173	278.34	C16H22O4
	Phthalic acid, isobutyl propyl ester			264.32	C15H20O4
	Phthalic acid, isobutyl propyl ester			264.32	C15H20O4
	E-7-0ctadecene	1.22	16.448		
.	1-Doco.scne				
	Trichloroacetic acid, hexadecyl ester			387.8	C18H33Cl3O2
	oxalic acid, isobutyl tetradecyl ester	0.33	16.508	342.5	C20H38O4
	1-Decanol., 2-hexylOxalic acid,			158.28	C10H22O
	Cyclobutyl pentadecyl ester			380.6	C23H40O4
	2h- Azepin-2-one, hexahydro-1-methyl-Piperidine	0.20			
	3- bromomethyl				
	Metaramino1			167.2	C9H13NO2
	Hentriacontane	0.20	17.080	436.8	C31H64

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	N-Methylallylamine			71.12	C4H9N
	Sulfurous acid, butyl heptadecyl ester			82.08	H2O3S
	9, 15-Octa.decadienoic acid, methyl ester, (Z,Z)-				
	9,12-octadecadienoic methyl ester, (E,E)-	3.30	17.399		
	9,12-octadecadienoic acid (Z,Z)-, methyl ester				
	9-octadecenoic acid (Z)-, methyl ester			296.5	C19H36O2
	9-octadecenoic acid (Z)-, methyl ester	5.22	17.451	296.5	C19H36O2
	9-0ctadecenoic acid -, methyl ester (Z)-			296.5	C19H36O2
	Pentadecanoic acid, methyl ester			256.42	C16H32O2
	Pentadecanoic acid, methyl ester	0.95	17.689	256.42	C16H32O2
	Pentadecanoic acid, methyl ester			256.42	C16H32O2
	Heneicosane			296.6	C21H44
	Hexadecane Oxalic acid,	0.52	17.778		
	6-ethyloct-3-yl hexyl ester			426.7	C26H50O4
	2-Butenediamide 1 2-methyl-(Z)- 2-Propenamide				
	N-11-cyclohexylethy 1)- Iron	0.18	18.068		
	Tetracarbonyl-2-(dimethylamino)ethylphosphine			274.01	C8H13FeNO4P+
	Fumaramic acid			115.09	C4H5NO3
	Sulfurous acid, pentyl tridecyl ester	0.23	18.149	334.6	C18H38O3S
	Sulfurous acid, hexadecyl pentyl e ster				
	2H-Azepin-2-one, hexahydro-7-methyl.-			127.18	C7H13NO
	2-Amino-1~(o-methoxyphenyl)propane	0.12	18.276		
	Uramil-N,N-diacetic acid			259.17	C8H9N3O7
	1-Alanine, N-valeryl-, nonyl ester				
	Methanesulfonam1de, N,N-dimethyl	0.55	18.922		
	Methanesulfonamide, N,N-dimethyl-				
	6-Tetradecanesulfonic acid, butyl ester			334.6	C18H38O3S
	Cyanoacetylurea	0.12	19.940	127.1	C4H5N3O2
	Butanal, 3-methyl-			86.13	C5H10O
	Methylpent-4-enylamine			99.17	C6H13N
	3-Methoxyamphetamine	0.09	22.517	165.23	C10H15NO
	3-Methoxyamphetamine			165.23	C10H15NO

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	Atomoxetine	0.12	23.208	255.35	C17H21NO
	1,4-Benzenedicarboxamide, N,N'-bis (2-hydroxy-1-methyl-2-phenylethyl)			432.5	C26H28N2O4
	p-Hydroxynorephedrine			167.2	C9H13NO2

Source: Organic compounds are identified by GC-MS (GC MS 5975 C Agilent), Mass spectrum, chromatogram, and datasheet provided by Bureau Veritas Consumer Products Services (I) Pvt. Ltd. Report Number: 7816-075-0003, 0004

3.3.4 Peaks data representation of chloroform extract of Graviola seeds:

The chloroform extract of Graviola seeds GC - MS chromatogram revealed 38 peaks exhibit three best hits indicating the presence of around one hundred and fourteen compounds (Figure 6). Total GC-MS chromatogram run time was 28minutes, three types of chemical compounds of each peak and their details were given in Table 5. The 1 N-Cyc1openta [1, 3] Cyclopropa [1, 2] benzene, octahydro-7-methyl-3-methylene-4 (1-methylethyl) -, [3aS- (3a. alpha.,3b.beta.,4.beta.,7.alpha.,7 aS·))-, 1.,6-Cyclodecadiene, 1-methyl-5-methy1ene-8-(l-methylethyl)-[S-(E, E, epi-Bicyclosesquiphellandrene compounds showed a moderate percentage of peak areas (14.65%) of the chromatogram. Whereas the Phthalic acid, cyclohexylmethyl butyl ester, 9,12-0ctadecadienoic acid (Z,Z)-, methyl ester, 9-0ctadecenoic acid (Z)-, methyl ester, Acetamide, 2-(4-iodo-lB-pyrazol-l-yl)-N-(4-ethoxycarbcnyl)phenyl- appeared more than the minimum percentage of peak areas (5.30%) of chromatogram. While the highest percentage of peak areas of chromatograms were noticed as Bicyclo [7.2.0] undec-4-ene, 4,11,11 trimethyl-8-methylene- (20.69%). In addition to these, a lot of other chemical compounds identified in trace amounts were notified in less than 5.0% peak area counts in the methanol extract of Graviola seeds. A list of all the bioactive compounds is mentioned with respective peak areas in Table. 3 corresponding to the GC-MS chromatogram Figure 2. The high peak area analytes of GC-MS chromatograms were ascertained in Table 4 alongside their chemical nature and pharmacological activities.

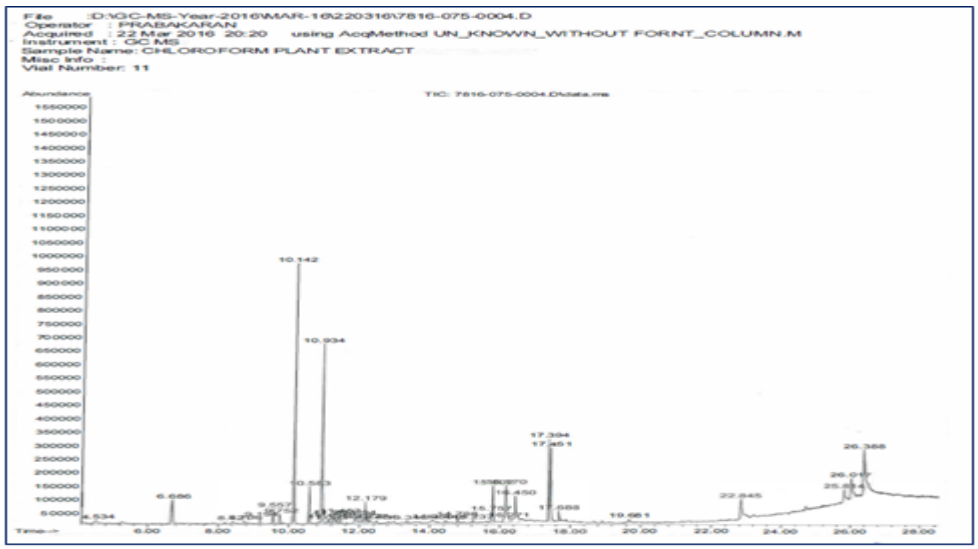

Figure No.2. GC-MS Chromatogram of chloroform extracts of Graviola seeds

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Table No. 3: GC-MS analysis of bioactive compounds of chlotroform extract of Gishta seeds.

Peak	Compound name	Retention time	Molecular weight	Molecular formula
	Benzenemethano:, .alpha.-(1-aminoe thyl}-, {H*,SA)-(.Present/-.}-	4.533		
	1-0ctadecanamine, N-rnethyl-			
	3-Methyl-3,5--{cyanoethyl.)tetrahyd ro-4-□tiopyranone			
	Naphthalene	6.687	128.169	C10H8
	Azulene		128.169	C10H8
	Azulene		128.169	C10H8
	2-Amino-1-(o-hydroxyphenyl)propane	8.418		
	Benzenemethar:ol, . alpha, -{ 1-amin oe ;:hyl)-, (R',S*)-(.Pt/-.)			
	Phenylephrine		167.2	C9H13NO2
	1-[.alpha.-{l-Adamantyl)benzyliden e]thiosemicarbazide	8.737		
	(-}-Norephedrine		151.21	151.21
	(1S,2R)-{Present}-Norephedrine		187.66	C9H14ClNO
	Phenylephrine	9.190	167.2	C9H13NO2
	Cathinone		149.19	C9H11NO
	Metaramino1			
	Copaene	9.554	204.35	C15H24
	.alfa,-Copaene			
	Copaene		204.35	C15H24
	cis-muurola-3, 5-diene	9.755	204.35	C15H24
	Bicyclo[4.4.0] dec-1-ene, 2-isopropyl-5-methyl-9-methylene-		204.35	C15H24
	Naphthalene, l,2,3,4,4a,5,6,8a-oct ahydro-7-methyl-4-methylene-1 (1-methylethy1)-, (l.alpha,.4a.beta.,8 a.alpha.)-			
	Caryophyllene	10.141	204.35	C15H24
	Caryophyllene		204.35	C15H24
	Bicyclo [7.2.0] undec-4-ene, 4,11,11trimethyl-8-methylene-			
	Humulene	10.587	204.35	C15H24

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	Humulene		204.35	C15H24
	4-Terpinenyl acetate		196.29	C12H20O2
	1.,6-Cyclodecadiene, 1-methyl-5-methy1ene-8-(l-methylethyl)-[S-(E, E	10.936	136.23	C10H16
	1 N-Cyc1openta [1, 3] Cyclopropa [1, 2] benzene, octahydro-7-methyl-3-methylene-4(1-methylethyl)-,[3aS-(3a. alpha.,3b.beta.,4.beta.,7.alpha.,7 aS·))-			
	epi-Bicyclosesquiphellandrene		204.35	C15H24
	Propanamide	11.092	73.09	C3H7NO
	Phenethylaraine, p,alpha.-dimethyl			
	1-octadecanamine, N-methyl-		283.5	C19H41N
	3-Chloro-N-methylpropylamine	11.129	107.58	C4H10ClN
	J. -(3, 5-Dimethyl -1-adamantanoyl) semicarbazide			
	Acetamide, 2-chloro-		93.51	C2H4ClNO
	Acetamide, 2-chloro	11.248	93.51	C2H4ClNO
	p-Hydroxynorephedrine		167.2	C9H13NO2
	2,4-Dimethylamphetamine		163.26	C11H17N
	Phenol, 2,4-bis(1,1-dimethylethyl)	11.293	310.5	C22H30O
	Phenol, 2,4-bis(1,1-dimethylethyl)		310.5	C22H30O
	Phenol, 3,5-bis(1,1-dimethylethyl)		312.4	C21H28O2
	2, 4 -Dimethylamphetamine	11.456		
	1,8-octanediamine, N,N'-dimethyl-			
	3,5-Dimethylamphetamine		163.26	C11H17N
	N-(3-Methylbutyl)acetamide	11.895	129.199	C7H15NO
	(5S)-5-Methyl-3-[(2R,8R,13R)-2,8,13-trihydroxy-13-{(2R,5R)-5-[(1R)-1-hydroxytridecyl]oxolan-2-yl}tridecyl]furan-2(5H)-one		595.4	C35H64O7
	Diimidotricarbonic diamide		146.11	C3H6N4O3
	Piperidine, 3-phenyl	12.703	395.5	C25H24F3N
	Octodrine		129.24	C8H19N
	Metaraminol		167.2	C9H13NO2
	Cyclooctene, 3-(1-methyletheny:)-	12.177		
	Cyclohexane, 1, 5-diethenyl-3-methyl- 2--methylene-, (l,alpha.,3.alpha, ' 5. Alpha)			
	Butyl 6,9,12,15-octadecatetraenoat			

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	Metaraminol	12.236	167.2	C9H13NO2
	Phenethylamine, p, .alpha.-dimethyl			
	Benzeneethanamine, N-methyl-		135.21	C9H13N
	Pheny1ephrine	12.481	167.2	C9H13NO2
	2-Amino-1-{o-hydroxyphenyl)propane		151.21	C9H13NO
	Norpseudoephedrine		151.21	C9H13NO
	Tenamfetamine	13.343	179.22	C10H13NO2
	Cyclohexanol, 2 -(methylaminomethyl)-, trans-		143.23	C8H17NO
	2, 6-Dimethoxyamphetamine		341.27	C14H16F5NO3
	Phenylephrine	14.079	167.2	C9H13NO2
	Tenamfetamine		179.22	C10H13NO2
	Tenamfetamine		179.22	C10H13NO2
	2-Propenamide, N-(1-cyclohexylethyl	14.450	181.27	C11H19NO
	Fturazan-3-carooxamiae, oxime, 4-amino-N,N-dimethyl-			
	Metaraminol		167.2	C9H13NO2
	N-Aminomorpholine	14.799	102.14	C4H10N2O
	N-Methyl-2-phenyl-1-propylamine		149.23	C10H15N
	Tetraacetyl-d-xylonic nitrile		343.29	C14H17NO9
	1,2-Benzenedicarboxylic acid, dipropyl ester	15.237	250.29	C14H18O4
	Didodecyl phthalate		502.8	C32H54O4
	Phthalic acid, 2-cyclohexylethyl butyl ester		332.4	C20H28O4
	Tocainide	15.757	192.26	C11H16N2O
	2-Amino-1-(o-methoxyphenyl)propane		165.23	C10H15NO
	p-Hydroxyamphetamine		151.21	C9H13NO
	Hexadecanoic acid, methyl ester	15.802	270.5	C17H34O2
	Hexadecanoic acid, methyl ester		270.5	C17H34O2
	Pentadecanoic acid, 14-methyl-, methyl ester		270.5	C17H34O2
	Diamyl phthalate	16.173	306.4	C18H26O4
	1,2-Benzenedicarboxylic acid, buty1 2-methylpropyl ester			
	Phthalic acid, cyclohexylmethyl butyl ester		318.4	C19H26O4
	lH-Indole-3-ethanamine, 6-fluoro-. beta.-methyl-	16.270		
	Phenylephrine		167.2	C9H13NO2

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	3-Methoxyamphetamine		165.23	C10H15NO
	9-Eicosene, (E) -	16.448	280.5	C20H40
	1-Docosene		308.6	C22H44
	Trichloroacetic acid, hexadecyl ester		387.8	C18H33Cl3O2
	Methyl 10-trans,12-cis-octadecadienoate	17.392	294.5	C19H34O2
	8,11-0ctadecadienoic acid, methyl ester			
	9,12-0ctadecadienoic acid (Z,Z)-, methyl ester			
	6- 0ctadecenoic acid, methyl ester, (Z)-	17.451		
	9- 0ctadecenoic acid, methyl ester, (E) -			
	9-0ctadecenoic acid (Z)-, methyl ester			
	Methyl stearate	17.689	298.5	C19H38O2
	Tridecanoic acid, 12-methyl-, methyl ester		242.4	C15H30O2
	Tetradecanoic acid, 12-methyl-, methyl ester		242.4	C15H30O2
	Acetamide, 2,2,2-trichloro-	19.860	162.4	C2H2Cl3NO
	2-Propen-1-amine, 2-bromo-N-methyl		150.02	C4H8BrN
	2-Heptanol, 6-amino-2-methyl-		145.24	C8H19NO
	9-0ctadeccnamide, { Z) -	22. 844		
	9-0ctadeccnamide, { Z) -			
	9-0ctadeccnamide, { Z) -			
	2-Ethylacridine	25.816	207.27	C15H13N
	Tris(tert-butyldirmethylsilyloxy)arsane			
	Arsenous acid, tris(trimethylsilyl) ester		342.49	C9H27AsO3Si3
	Benzo[h}quinoline, 2,4-dimethyl-	26.016	252.27	C15H12N2O2
	2-Ethylacridine		207.27	C15H13N
	5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan.		207.14	C8H5N3O4
	Benz(b] J.,4-oxazepine-4(5H)-thione, 2, 3-dihydro-2 1 8-dihydro-2, 8-methyl-	26.388		
	4-Methyl-2-trimethylsilyloxy-aceto phenome			
	Acetamide, 2-(4-iodo-lB-pyrazol-l-yl)-N-(4-ethoxycarbcnyl)phenyl-			

Source: Organic compounds are identified by GC-MS (GC MS 5975 C Agilent), Mass spectrum, chromatogram, and datasheet provided by Bureau Veritas Consumer Products Services (I) Pvt. Ltd. Report Number: 7816-075-0003, 0004

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Table No. 4: Pharmacological activities of major phytocomponents in the solvent extract of Gishta seeds.

Bioactive compounds	**Chemical Nature (Obasi , L et al., 2010)**	**Pharmacological Activities (Prashant Tiwari et al., 2011)**
Caryophyllene	bicyclic sesquiterpene	Antagonistic properties of cancer, tumor, inflammatory, microbial, and oxidant properties. Soothe anxiety and pain, lower cholesterol, control osteoporosis, and seizures.
Bicyclo [7. 2. 0] undec-4-ene, 4, 11, 11- trimethyl-8-methylene	Terpene	Act against to cancer, tumor, inflammation, microorganisms and oxidants. soothe anxiety and pain, lower cholesterol, control osteoporosis, and seizures.
9, 15-Octa.decadienoic acid methyl ester,	linoleic acids and derivatives glycosides.	Control cholesterol, act against Nematodes, arthritis, acne, eczema, histamine, androgeny, coronary, liver protective and insect repellant.
(5S)-5-Methyl-3-[(2R,8R,13R)-2,8,13-trihydroxy-13-{(2R,5R)-5-[(1R)-1-hydroxytridecyl]oxolan-2-yl}tridecyl]furan-2(5H)-one	Alkaloids – long chain fatty acids with terminal lactone ring	Antibacterial, Antiseptic, Candidacies, Cytotoxic, Dopamine-Adenylate-Cyclase-Inhibitor, Fungicide, Hypotensive, Insecticide, Mycoplasmicide and Pesticide
(Z, Z) - 9,12-octadecadienoic methyl ester,	Linoleic acid	Control cholesterol, act against Nematodes, arthritis, acne, eczema, histamine, androgyny, coronary, liver protective and insect repellant.
1H-Cyclopenta [l, 3] cyclopropane [l, 2] Benzene	Cyclopropanes; Alkaloids	Act against microorganisms, diarrhea and helminthic
octahydro-7-methyl-3-methy lene-4-(1-methylethyl) -,	Sesquiterpenes	Act against inflammation, microorganisms, cancer, tumor and oxidants. soothe anxiety and pain, lower the cholesterol, control osteoporosis, seizures.
[3aS-(3a. alpha., 3b. beta., 4. beta., 7. alpha., 7 aS*)]-Bicyclo [4. 4. 0] dec-l-ene	Terpene	Act against inflammation, microorganisms, cancer, tumor and oxidants. soothe anxiety and pain, lower the cholesterol, control osteoporosis, seizures.
2-isoprop yl-5-methyl-9-methylene-. gamma. – Muurolene	sesquiterpene, a carbo bicyclic compound	Act against inflammation, microorganisms, cancer, tumor and oxidants. soothe anxiety and pain, lower the cholesterol, control osteoporosis, seizures.

Source: Dr Dukes Phytochemical and Ethnobotanical Databases

4. Discussion:

New inorganic and organic based treatments should be developed to address the problems like antibacterial resistance and cancer (Yee Y et al., 2009; Khan A. Q et al,. 2011). Chloroform extracts of Ghista confirmed the presence of alkaloids, fixed oils, flavonoids, polyketides, terpenoids, steroids and xanthoproteins, whereas the methanolic extracts showed the presence of alkaloids, carboxylic acids, fixed oils, flavonoids, phenols, polyketides, terpenoids, quinones, resins, saponins, steroids, tannins, xanthoproteins and glycosides(Amtaghri Smail et al., 2022). Results are

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more or less consistent with that of Usunobun et al. (2014). Powerful analgesic drugs can be prepared from alkaloids and they have wonderful physiological effect on human (Kam and Liew, 2002; Aremu et al., 2016). Hypercholesterolemia, hyperglycaemia, anticancer, antioxidant, anti-inflammatory, weight loss and antifungal properties were shown by saponins (Tijjani et al., 2012). Saponins have haemolytic activities (Mensah et al., 2013). Triterpenoids (C30) have many medicinal properties (Martinez et al., 2008). Mensah et al., 2013, published the disease curing nature of Flavonoids. Tannins exhibit antimicrobial, antitumor properties and cure lung disorders and certain fevers (Gills, 1992). HIV replication controlling ability of tannins was reported by (Tijjani et al., 2012). Tannins are used in treating ulcers (Adegboye et al., 2008, Aremu et al., 2017)). Tannins inhibit protein synthesis (Shimada, 2006). Phenols that are obtained from different plants have medicinal properties (Adesuyi et al., 2011).

Results of this investigation showed that solvents extracts of Gishta seeds are very rich in phytochemicals, despite of slight difference in possessing phytochemicals of the three parts. The preliminary screening tests are expected to be useful in the detection of the bioactive principles, exploration of novel compounds and facilitate the qualitative separation of active compounds. Annona spp. is used in folkloric treatment of many health hazards (Gajalakshmi et al., 2012).

GC-MS analysis of a methanol extract of Graviola-seeds showed two hundred and ten chemical compounds of seventy peaks of the chromatogram and chloroform extract showed one hundred and fourteen chemical compounds of thirty-eight peaks of chromatogram. The Cyclo-dec-lene- Muurolene (42.53%) is the highest compound, Bicyclo undec-4-ene (13.39%) tetradecane (18.21%) found in moderate amounts and Decenoic Acid (8.5%) is the lowest chemical compound, in addition to these Caryophyllene is also found in significant amounts in the Graviola seeds extract. Earlier researchers reported the pharmacology of these chemical substances in the oils of clove, cannabis, rosemary, and hops (Alamgir A.N.M, 2018). 9,15-Octa.decadienoic acid methyl ester and (Z, Z)-9, 12-octadecadienoic methyl ester were identified in Graviola seeds extract comes under the group based on their chemical nature considered as lineolic acids and derivatives of glycosides, previous researchers attributed pharmacological activities to methyl linoleate (Cruz ASP, 2008; Wang GS, 2010; Shaibani TRMA et al,. 2009; Cowan MM, 1999; Kumar R et al., 2010). Linoleic acids are also found in other plant species such as clove, garlic, jasmine, white mustard, parsle and witch hazel (FDB012761, 2021). [3aS-(2-isoprop yl-5-methyl-9-methylene-.gamma. -Muurolene, 3a. alpha., 3b. beta., 4. beta., 7. alpha., 7 aS*)]-Bicyclo [4. 4. 0] dec-l-ene, octahydro-7-methyl-3-methy lene-4-(1-methylethyl) -, Caryophyllene and Bicyclo [7. 2. 0]undec-4-ene, 4,11,11- trimethyl-8-methylene are found in the Graviola seeds extract comes under bicyclic sesquiterpene shows various pharmacological properties (Sutar N et al., 2010; Eloff JN, 1998; Audu SA et al., , 2007; Prashant Tiwari et al., 2011). Caryophyllene has antiinflammatory properties (Cho JY et al., 2007 and Javed H et al., 2016). Caryophyllene fights against dental plaque causing bacteria (Pieri FA et al., 2016) and also other microorganisms (Swamy MK et al., 2016). They can also fight against the complications of multiple sclerosis and malfunctioning of immune system (Yamaguchi M and Levy R, 2016). They have local anaesthetic properties (Dias DS et al., 2014). It can produce endorphins without being addictive (Russo EB and Taming THC, 2011; Katsuyama S et al., 2013). 1H-Cyclopenta [l, 3] cyclo-propa [l, 2] benzene is a bioactive compound found in major portions in Gishta (Graviola) seed extract, which exhibits cyclopropane's chemical nature comes under Alkaloids. A lot of research revealed Alkaloids possess a good number of pharmacological activities mentioned as antimicrobial, antidiarrhoeal, and anthelmintic (Ncube NS et al., 2008; Remington JP. Remington, 2005; Handa SS et al., 2008; Das K et al., 2010). Further research like isolation and characterization of different phytochemical constituents may help to discover the new drugs (G. Sarkissian, J. et al., 2004; Ma, Q.Z et al., 2011).

It is clear that the plant extract mediated biosynthesis is amenable, effective, economical, ecofriendly and has no side effects for human therapeutic use. The green nanoparticles synthesis is a recently emerged viable method and a great alternative to the existing procedures (Chung IM, 2016; Ghotekar, S et al., 2019; Mukesh N. Kher et al., 2024).

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5. Conclusions:

Ag-NPs of Gishta seed extracts were synthesised to study phytochemical and GC-MS studies. Results of this investigation showed that solvents extracts of Gishta seeds are very rich in phytochemicals. Phytochemical profile of chloroform extracts of Gishta revealed and highlighted the presence of Alkaloids, Fixed oils, Flavonoids, Phytoketoids, Terpenoids, Quinones, Steroids and Xanthoproteins. Whereas methanolic extracts of Gishta recorded the presences of phyto-consituents like Carboxylic acids, Flavonoids, Phenols, Phytoketoids, Fixed oils, Terpenoids, Alkaloids, Resins, Steroids, Quinones, Tannins, Xanthoproteins, Glycosides and Saponins. These constituents may be accountable for the efficient capping and chelating agent of nanoparticles and also responsible for exhibiting biological and bio efficacy activities. The Ag-NPs had shown very prominent results and UV-VIS spectral reports exposed the absorption peak and multi dispersed nature of the plant extract under study. GC-MS analysis of a methanol extract of Graviola-seeds showed two hundred and ten chemical compounds of seventy peaks of the chromatogram and chloroform extract showed one hundred and fourteen chemical compounds of thirty-eight peaks of chromatogram. The Cyclo-dec-lene-Muurolene (42.53%) is the highest compound, Bicyclo undec-4-ene (13.39%) tetradecane (18.21%) found in moderate amounts and Decenoic Acid (8.5%) is the lowest chemical compound, in addition to these Caryophyllene is also found in significant amounts in the Graviola seeds extract.

References:

1. A.N.M. Alamgir. Therapeutic Use of Medicinal Plants and their Extracts: Volume 2: Phytochemistry and Bioactive Compounds Volume 74 of Progress in Drug Research, Springer, 2018. p. 173-176. [cited 2021 March 15] Available from: https://books.google.co.nz/books?id=2LZhDwAAQBAJ&source=gbs_navlinks_s
2. Adegboye MF, Akinpelu DA & Okoli AI 2008. The bioactive and phytochemical properties of Garnicial kola Hackel seed extract on some pathogens. Afr. J. Biotechn., 72(1): 3934-3938.
3. Adesuyi AO, Anokwuru CP, Esiaba I, Ajibaye O,. Polyphenolic content and antioxidant activity of Hibiscus sabdariffa calyx. Res J Med Plant. 2011;5:557–566.
4. Amtaghri Smail, Akdad Mourad, Slaoui Miloudia and Eddouks Mohamed, Traditional Uses, Pharmacological, and Phytochemical Studies of Euphorbia: A Review, Current Topics in Medicinal Chemistry 2022; 22(19).https://dx.doi.org/10.2174/1568026622666220713143436.
5. Aremu, MO, Ibrahim, H & Ekanem BE 2016. Effect of processing on in–vitro protein digestability and anti–nutritional properties of three under–utilized legumes grown in Nigeria. Br. Biotechnol. J., 14: 1–10.
6. Aremu, MO, Audu, SS & Gav BL 2017. Comparative review of crude protein and amino acid composition of some leguminous seeds grown in Nigeria. Int. J. Sci., 6: 88–97.
7. Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). Life Science Journal 2007; 4(4): 75-79.
8. Cho JY, Chang HJ, Lee SK, Kim HJ, Hwang JK, Chun HS. Amelioration of dextran sulphate sodium-induced colitis in mice by oral administration of beta-caryophyllene, a sesquiterpene. Life Sci. 2007;80(10):932-9. doi: 10.1016/j.lfs.2006.11.038, PMID 17188718.
9. Chung IM, Park I, Seung-Hyun K, Thiruvengadam M, Rajakumar G. Plant-Mediated Synthesis of Silver Nanoparticles: Their Characteristic Properties and Therapeutic Applications. Nanoscale Res Lett. 2016 Dec;11(1):40. doi: 10.1186/s11671-016-1257-4. Epub 2016 Jan 28. PMID: 26821160; PMCID: PMC4731379.
10. Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews 1999; 12(4): 564-582.
11. Cruz ASP. Anthelmintic effect of Solanum lycocarpum in mice infected with Aspiculuris tetraptera. The journal of American science 2008; 4(3): 75-79.
12. Debela A 2002. Manual for phytochemical screening of medicinal plants. Ethiopian

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 658-681 | ISSN:2251-6727

Health and Nutritional Research Institute, Addis Ababa, Ethiopia, pp. 35-47.

13. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacology 1998; 60: 1–8.
14. G. Sarkissian, J. Keegan, E. Du Pasquier, J.-P. Depriester & P. Rousselot., 2004. The Analysis of; Tires and Tire Traces using FTIR and Py-GC/MS, Canadian Society of Forensic Science Journal, 37:1, 19-37, DOI: 10.1080/00085030.2004.10757566.
15. Gajalakshmi, S., Vijayalakshmi, S., & Devi Rajeswari, V. (2012). Phytochemical and pharmacological properties of Annona muricata: a review. International Journal of Pharmacy and Pharmaceutical Sciences, 4(2), 3-6.
16. M.V. Raju, K. Maria Das, Md. B. Wasim Akram, T.L. Rishi Vardhan and Hepsibah Palivela, 2019, Water Quality Evaluation of Aquaculture Practices in Coastal Area of Bhimavaram at West Godavari District, Andhra Pradesh, JARDCS,Vol.11, 11- Special Isuue, Pages: 1088-1094. 10.5373/JARDCS/V11SP11/20193140.
17. Ghotekar, S., Pansambal, S., Pawar, S.P. et al. Biological activities of biogenically synthesized fluorescent silver nanoparticles using Acanthospermum hispidum leaves extract. SN Appl. Sci. **1**, 1342 (2019). https://doi.org/10.1007/s42452-019-1389-0
18. Gills LS 1992. Ethno- medical uses of plants in Nigeria. University of Benin Press, Nigeria pp 276.
19. Gomathi, D., Kalaiselvi, M., Ravikumar, G. et al. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. J Food Sci Technol **52,** 1212–1217 (2015). https://doi.org/10.1007/s13197-013-1105-9.
20. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. International centre for science and high technology, Trieste, 2008, 21-25.
21. Harbone JB 1973. Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. Chapman and Hall Ltd, London, pp. 49-188.
22. Harikrishna Ramaprasad Saripalli., 2004. Antibacterial activity of Croton bonplandianum, Moringa pterigosperma and Physalis minima. M.Phil Thesis, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India, pp. 23.
23. http://www.blackherbals.com/graviola.htm "Graviola (Soursop)". Blackherbals. Retrieved 30 January, 2019.
24. https://www.frontiersin.org/articles/10.3389/fmolb.2020.00193,DOI=10.3389/fmolb.2020.00193, ISSN 2296-889X.
25. Katsuyama S, Mizoguchi H, Kuwahata H, Komatsu T, Nagaoka K, Nakamura H, Bagetta G, Sakurada T, Sakurada S. Involvement of peripheral cannabinoid and opioid receptors in β-caryophyllene-induced antinociception. Eur J Pain. 2013;17(5):664-75. doi: 10.1002/j.1532-2149.2012.00242.x, PMID 23138934.
26. Khan A. Q., Bury J. P., Brown S. R., Riley S. A., Corfe B. M. Keratin 8 expression in colon cancer associates with low faecal butyrate levels. BMC Gastroenterology. 2011;**11**(1):p. 2. doi: 10.1186/1471-230X-11-2.
27. Kumari, G. V. R., Raju, M. V., Kumar, M. S., Das, K. M., & Satyanarayana, D. (2023, June). Solid waste management: A case study. In AIP Conference Proceedings (Vol. 2766, No. 1). AIP Publishing.
28. Kumar R, Sharma RJ, Bairwa K, Roy RK, Kumar A. Pharmacological review on natural antidiarrhoel agents. Der Pharma Chemica 2010; 2(2): 66-93.
29. Ma, Q.Z., Wu, F.J., Zhang, D.Q., Peng, W.X., 2011. Py-GC-MS Analysis on Benzene-Alcohol Extractives of Phyllostachys pubescens for Biomedical Engineering. KEM 480–481, 211–214. https://doi.org/10.4028/www.scientific.net/kem.480-481.211
30. Martinez MJA, Lazaro RM, del Olmo LMB & Benito PB 2008. Anti-infectious activity in the anthemideae tribe. In: Atta – Ur- (ed.) Studies in Natural Product Chemistry, Elsevier, pp. 44-56.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 658-681 | ISSN:2251-6727

31. Mensah JK, Ihenyen JO & Okhiure MO 2013. Nutritional phytochemical and antimicrobial properties of two wild aromatic vegetables from Edo State. J. Natural Products and Plant Resou., 3(9): 8-11.
32. Mariadas, K., Rao, T. S., Raju, M. V., & Kumar, M. S. (2024). A Study On The Fluoride Content Of The Groundwater In The Gurazala Division Of The Palnadu District, Andhra Pradesh, India. Journal of Advanced Zoology, 45(3). pp.631-636.
33. Mukesh N. Kher, Sandip P. Dholakia, Dipen K. Sureja, Vaibhav D. Bhatt, Nirav V. Patel (2024). Chapter 5 - Green synthesis of silver nanoparticles, characterization techniques and biological activities, Editor(s): Mital Kaneria, Kalpna Rakholiya, Chukwuebuka Egbuna, Nanotechnology and In Silico Tools, Elsevier, Pages 61-74, ISBN 9780443154577, https://doi.org/10.1016/B978-0-443-15457-7.00006-X.
34. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7 (12): 1797-1806.
35. Pieri FA, Souza MC, Vermelho LL, Vermelho ML, Perciano PG, Vargas FS, Borges AP, da Veiga-Junior VF, Moreira MA. Use of β-caryophyllene to combat bacterial dental plaque formation in dogs. BMC Vet Res. 2016;12(1):216. doi: 10.1186/s12917-016-0842-1, PMID 27716286.
36. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur (2011). Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. Vol 1 Issue 1:, pp 98- 106, http://www.ipharmsciencia.com.
37. Raju, M. V., Neelakanta Rao, L., Mariadas, K., Siva Jagadish Kumar, M., & Ramesh Babu, S. (2019). A study on metals recovery from the waste water effluents in electroplating industry. International Journal of Civil Engineering and Technology (IJCIET), 10(2), 1020-1040.
38. Remington JP. Remington: The science and practice of pharmacy, 21st edition, Lippincott Williams & Wilkins, 2005, 773-774.
39. Raju, M. V., Palivela, H., Mariadas, K., & Babu, S. R. (2019). Assessment of physico-chemical and biological characteristics and suitability study of lake water: a model study. Technology, 10(01), 1431-1438.
40. Ruppercht, J.K., Chang, C.J., Cassady, J.M and McLaughlin, J.L.,1982. Heterocycles, 24:1197-1201.
41. Raju, M. V., Mariadas, K., Palivela, H., Ramesh Babu, S., & Raja Krishna Prasad, N. (2018). Mitigation plans to overcome environmental issues: A model study. International Journal of Civil Engineering and Technology, 9(10), 86-94.
42. Russo EB. Taming THC: potential cannabis synergy and phyto-cannabinoid - terpenoid entourage effects. Br J Pharmacol. 2011; 163: 1351.
43. Raju, M. V., Mariadas, K., Kumari, G. V. R., Kumar, M. S. J., & Babu, S. R. (2018). An extensive examin quality and so characterstices i andhra pradesh cor at amaravati b. Technology (IJCIET), 9(11), 2113-2125.
44. Saripalli, Harikrishna & Dixit, Prasanna., 2016. Studies on Morphological Features and Biological Activities of the Genus Annona of Ethiopia, N. E. Africa with a Special Emphasis on Graviola: A Review. International Journal of Science and Research (IJSR). 5. 821-827.
45. Sermakkani M. and Thangapandian. V. GC-MS Analysis of Cassia Italica leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research Vol 5, Issue 2, 2012; 90-94.
46. Shimada T. Salivary proteins as a defense against dietary tannins. J Chem Ecol. 2006 Jun;32(6):1149-63. doi: 10.1007/s10886-006-9077-0. Epub 2006 May 23. PMID: 16770710.
47. Sofowora A 1993. Screening Plants for Bioactive Agent: Medicinal Plants and Traditional Medicine in Africa 2nd Edn. Spectrim Books Ltd., pp. 134-156.
48. Sutar N, Garai R, Sharma US, Sharma UK. Anthelmintic activity of Platycladus orientalis leaves extract. International Journal of Parasitology Research 2010; 2(2): 1-3.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 658-681 | ISSN:2251-6727

49. Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evid Based Complement Alternat Med. 2016; 3012462. doi: 10.1155/2016/3012462, PMID 28090211.

50. Tijjani MA, Abdurahman FI, Buba SW, Mada GI, Akan JC, Aji BM & Abdullah AS 2012. Chemical and extract of Pilliotigma thonningii Schum (camel foot). J. Chem. and Pharmac. Res., 49(5): 2409-2414.

51. Vutukuru, S. S., Asadi, S. S., Vasantha, R. B. V. T., & Raju, M. V. (2012). Plankton biodiversity as indicators of the ecological status of River Moosi, Hyderabad, India. International Journal of Earth Science and Engineering, 5(3), 587-592.

52. Trease GE and Evans WC 1989. Pharmacognosy, 13th Edn. Bailliere Tindall, London, pp. 134-156. https://doi.org/10.1016/j.chemosphere.2022.135652

….

53. Usunobun U, Okolie NP, Anyanwu OG & Adebegi AJ 2014. Phytochemicals screening and proximate composition of Annona muricata leaves. Euro. J. Botany Plant Sci. and Pathol., 2(1): 18-28.

54. Wang GS, Han J, Zhao LW, Jiang DX, Liu YT, Liu XL. Anthelmintic activity of steroidal saponins from Paris polyphylla. Phytomedicine 2010; 17: 1102-1105.

55. Yamaguchi M, Levy R. β-caryophyllene promotes osteoblastic mineralization and suppresses osteo clasto genesis and adipogenesis in mouse bone marrow cultures in vitro, 2016.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5228512.

56. Yee Y., Tan V. P. Y., Chan P., Hung I. F., Pang R., Wong B. C. Epidemiology of colorectal cancer in Asia. Journal of Gastroenterology and Hepatology. 2009; **24**(12):1810–1816.doi: 10.1111/j.1440-1746.2009.06138. x.