Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

# Pharmacognostic, Phytochemical Evaluation and Antioxidant Activity of *Onosma Bracteatum*

**Sweety Jaggi khatri, Bhaskar Kumar Gupta**

School of Pharmacy, Peoples University, Bhopal

***(Received: 16 March 2025 Revised: 20 April 2025 Accepted: 01 May 2025)***

**KEYWORDS**

O. bracteatum, Secondary Metabolites, TLC, Antioxidant

**ABSTRACT:**

The aim of study is to isolate and quantify compound and evaluation of Phytochemical & antioxidant potential of extract and fractions of leaves of O. bracteatum. The information obtained from preliminary phytochemical screening will be useful in finding out the genuine of the drug. The result of phytochemical screening of powder drug, alcohol, aqueous extract of Onosma bracteatum leaf are presented in the phytochemical screening indicated varying quality of alkaloids, carbohydrate, glycoside, sterol, saponins, protein, mucilage, terpenoid, flavonoid, phenol, protein and amino acids in the leaf extract. The phytochemical profile revealed that the methanolic and ethanolic extract contained aldehydes, carbohydrate, glycoside, sterol, saponins, protein, mucilage, tannins, terpenoids and phenols. The antioxidant activities of different extracts were indexed by the DPPH radical scavenging activity.Different extracts possessed varying free radical scavenging activities. Among the extracts tested, methanolic extract was the most potent extract with an IC50 value of 21.76 µg/mL with percentage inhibition of 85.46. Compared to methanolic extract, although ethyl acetate and ethanolic extract exhibited a lower radical scavenging activity with respective IC50 values of 28.31 µg/mL and 33.49 µg/mL with percentage inhibition of 76.37 and 64.75 respectively. IC50 value of methanolic extracts had significantly the same radical scavenging activity as that of ascorbic acid (IC50¬18.21 µg/mL).

## INTRODUCTION

Medicinal plants are in sought after as a primary source for lead molecules in drug discovery. According to one report statistically defined chemical space is similar for natural products and drugs, while combinatorial compounds and drugs do not share similar space (Lachance *et al.*, 20012).

The recent advancement in science has opened up new avenues and facilitated search of the new therapeutic agents from medicinal plants that are used in traditional medical practices. Plant selection is corner stone in drug discovery and the systematic approach of exploring traditional, ethno-medicinal, ethno-pharmacological or ethno-botanical literature is usually opted for (Rajapara *et al.,* 2021).

The present study is one such effort and investigates a traditionally valued indigenous medicinal plant Onosma bracteatum Wall. (Family: Boraginaceae) commonly known as ‘Gaozaban’/ ‘Gojihva’). The plant is valued as a tonic in building the body immune resistance, spasmolytic and diuretic and is indicated in the treatment of asthma, bronchitis, rheumatism, irritation of bladder, syphilis, leprosy, wound and skin diseases (Pattanayak, S. (2019)).

Documented records on *O. bracteatum* is suggestive of its antioxidant, anti-inflammatory and antiallergic, antidiarrhoeal, psycho-Immunomodulatory, wound healing, antibacterial, analgesic, anticancer and anti-ageing activities. In the event of inconsequential literature on both pharmacological and phytochemical aspects we here propose bioactivity guided extraction of *O. bracteatum* with specific reference to evaluation of its antioxidant and cyototoxic potential (Gasimova *et al.*, 2024).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

Traditionally, genus Onosma L. plants are used as a stimulant in rheumatism, bladder pain, kidney irritation, palpitation of heart and roots for their diuretic, cooling, astringent and demulcent action. While in India, it is used in the treatment of hypertension, fever and nervous conditions. In Turkey, these plants are used to treat inflammatory disorders such as tonsillitis, hemorrhoids and bronchitis and pain.

**Collection & Authentication of the Plant Sample**

The whole plant of *Onosma bracteatum* were collected from Bhopal, MP, India, during the months of September and October 2022 and all the primary work done (washing, drying etc.).The plant materials were identified and authenticated by Dr. Saba Naaz, Department of Botany, Safiya Science college Bhopal. The herbarium of this specimen was kept in the department for further reference.

Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Parekh et. al., 2006). In this study, following plants were selected for their antibacterial activity against MDR pathogens and phytochemical analysis on the basis of their medicinal use:

*Onosma bracteatum* plant was washed with cold distilled water, and then dried under hot air at a temperature not exceeding 60°C in the shadow. The samples were crushed properly by metal mortar until a fine homogeneous powder was obtained, kept in paper bags under free humidity conditions (Cipriano RC 2001)

**Organoleptic Characters**

Organoleptic evaluation can be done, by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. Organoleptic characters (External appearance) such as shape, size, colour, odour, taste and leaf structure like margin, Epidermis, stomata, fibres, etc. are evaluated. (Siddiqui *et al.*, 1995).

**Determination of Colour**

The untreated part of the drug was taken and colour of the drug was examined under sunlight.

**Determination of Odour**

A small portion of the drug was taken, slowly and repeatedly inhaled the air over the material and examined the odor.

**Determination of Taste**

For taste, a small portion of drug was taken on the tongue and find out the taste of drug. The parameters which are studied are moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble, extractive values, petroleum ether soluble extractive value, ethyl acetate soluble extractive value, acetone soluble extractive value, etc.

**Determination of Ash**

The residue remaining/left after incineration of the crude drug is designated as ash. The ash remaining following the ignition of medicinal plants is determined by three different methods which measures, total ash, acid-insoluble and water-soluble ash. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Bele A et al., 2011). The procedure recommended in Indian Pharmacopoeia (IP) .were followed for the determination of total ash, water-soluble ash, acid- insoluble ash, sulphated ash and loss on drying.

**Loss on Drying (LOD):**

It is used for determination of moisture content. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence, the moisture content of the drug should be determined and should also be controlled. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination.

**Determination of Foreign Organic Matter**

**Procedure**

An accurately weighed 100g of the plant material and spread it out in a thin layer. The plant material was detected by inspection with unaided eye or using a lens (6x). Then, the foreign matter was separated and weighed. The percentage yield of the foreign organic

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

matter was calculated with reference to the drug taken. The results obtained are presented in Table 1.

**Determination of Extractive Values**

Extractive value of crude drug estimated as per guidelines of IP 1996 and WHO Quality Control Methods for Medicinal Plant Materials 1998. These values are the indicatives of the approximate measures of their chemical constituents and the nature of the constituent present in the crude drug. Taking into consideration the diversity in chemical nature and the properties of content of drugs, various solvents are used for determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substance desired.

**Determination of methanol soluble extractive value**

An accurately weighed 5g of the air dried coarsely powdered drug was macerated with 100mL of methanol in a conical flask for 24 h, shaking frequently during the first 6h and allowed to stand for 18h. Thereafter, filter rapidly taking precautions against loss of methanol. 25mL, of the filtrate was evaporated to dryness in a china dish and dried at 105℃ and weighed. The percentage of methanol soluble extract with reference to the air-dried drug was calculated. The results obtained are presented in Table 1.

**Determination of Ethanol Soluble Extractive Value**

An accurately weighed 5g of the air dried coarsely powdered drug was macerated with 100mL of ethanol in a conical flask for 24 h, shaking frequently during the first 6h and allowed to stand for 18h. Thereafter, filter rapidly taking precautions against loss of ethanol. 25mL, of the filtrate was evaporated to dryness in a china dish and dried at 105 ° and weighed. The percentage of ethanol soluble extract with reference to the air-dried drug was calculated. The results obtained are presented in Table 1.

**Determination of Water Soluble Extractive Value**

The procedure adopted under ethanol soluble extractive was followed using water as a solvent. The results obtained are presented in Table 1.

**Determination of Chloroform soluble Extractive Value**

An accurately weighed 5g of the air dried coarsely powdered drug was macerated with 100mL of chloroform in a conical flask for 24h, shaking frequently during the first 6h and allowed to stand for 18h. Thereafter, filter rapidly taking precautions against loss of chloroform. 25 mL, of the filtrate was evaporated to dryness in a china dish and dried at 105° and weighed. The percentage of chloroform soluble extract with reference to the air-dried drug was calculated. The results obtained are presented in Table 1.

**Determination of Petroleum Ether Soluble Extractive Value**

The procedure adopted under ethanol soluble extractive was followed using petroleum ether as a solvent. The results obtained are presented in Table 1.

**Determination of Ethyl Acetate Soluble Extractive Value**

The procedure adopted under ethanol soluble extractive was followed using benzene as a solvent. The results obtained are presented in Table 1.

The leaves were shade dried, made into coarse powder and the powdered material was initially defatted with petroleum ether and then subjected to cold maceration process for 60 h using same proportions of mixture of methanol and water as solvent to prepare hydro-alcoholic extract of leaves (percentage yield 20.5% w/w with respect to dried powder). The extract was filtered and concentrated by rotary evaporator. For the preparation of different fractions method was used (Harbone, 1973).

**Extraction of Plant Material Using Maceration Procedure**

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

sample surface with the solvent system (Fabricant DS and Farnsworth NR; 2006).

After identification and authentication of the plant, leaves of the plant were collected for the experimental process. The leaves were shade dried, made into coarse powder and the powdered material was initially defatted with petroleum ether and then subjected to cold maceration process for 60h using same proportions of methanol as solvent to prepare methanolic extract of leaves (percentage yield 23.89% w/w with respect to dried powder). The extract was filtered and concentrated by rotary evaporator. For the preparation of different fractions method was used (Harbone, 1973).

**Preliminary Phytochemical Screening of Extract**

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. A brief summary of the experimental procedures for the various phytochemical screening methods for the secondary metabolites is shown in Table. After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests as shown in the Table to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction.

Preliminary phytochemical screening of the whole plant powder and/or crude drugs extracted in different solvents has been performed to detect the phytoconstituents like; alkaloid, aminoacid, carbohydrate, glycoside, mucilage, tannin, saponins, steroid, triterpenoid, Gums, fixed oils, fat, phenol and flavonoid were qualitatively analyzed by using the standard procedures. (**Harbone J.B 1998&Kokate C. K., (2008).**

**A). Alkaloids (**Harbourne, JB, 1984, Kokate CK, 1994 and Evans WC, 1997)

**B). Glycosides**

**I) Phytosterol** (Finar, 1967; Trease, 1989)

**J)Flavonoids**

**Qualitative Chromatograhic Analysis**

**Thin layer chromatography**

**Principle**

Chromatographic separations take advantage of the fact that different substances are partitioned differently between two phases, a mobile phase and a stationary phase. In thin layer chromatography, or TLC, the mobile phase is a liquid and the stationary phase is a solid absorbent.

TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction.

**Procedure**

TLC plates were prepared with a 50% solution of silica gel (stationary phase) adsorbed on glass plates. The plates were dried and were the activated at 110˚C for an hour. Specific solvent systems (mobile phase) and respective developing reagents for specific class of compounds based on the polarity of the solvents were prepared. Solutions of the extracts to be tested were also prepared using organic solvents. The extracts were spotted on the activated TLC plates using separate capillary tubes. The plates were then placed in glass chamber filled with specific solvents systems. The solvent system or mobile phase rises up the plates via capillary action carrying the components of the extracts with it. When the solvent system covers up about 80-90% of its path, the plates are removed from the chamber and dried. On drying, the plates are sprayed with specific developing agents. Different compounds are indicated by development of different colored spots on the plates. These spots were observed and the presence or absence of the compounds was concluded. Results are presented in table no. 4.26 – 4.29 (Atieno NR et al., 2013& Gamal MES. and Shehata ME 2014)

The solvent system and developing agents used for the study were as follows.

**For Alkaloids**

Solvent system: Ethyl acetate: Chloroform: Methanol (20:20:10)

Developing Agent: Dragendroff's Reagent

Observation: Orange spots indicate the presence of alkaloids.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

**For Tannins**
Solvent system: n Butanol: Acetic acid: Water (4:1:5)
Developing Agent: 10% FeCl3 in methanol and water (1:1)
Observation: Grey spots indicate the presence of tannins.

**For Flavonoids**
Solvent system: Ethyl acetate: Formic acid: Water (8:1:1)
Developing Agent: 15 ml 3% Boric acid + 5 ml 10% Oxalic acid
Observation: Fluorescent green spots in UV light indicate the presence of flavonoids.

**For Cardiac glycosides**
Solvent system: Chloroform: Methanol (9:1)
Developing Agent: Dinitrobenzoic acid
Observation: Brick red spots after incubation at 60 – $70^0$C for 5 minutes indicates the presence of cardiac glycosides.

**For Saponins**
Solvent system: Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8)
Developing Agent: 0.5 ml Anisaldehyde + 10 ml Glacial acetic acid + 85 ml
Methanol + 5 ml H2SO4
Observation: Red spots in visible light indicate presence of saponins.

**For Terpenes**
Solvent system: Ethyl acetate: Acetic acid: Formic acid: water (100:11:11:26)
Developing Agent: Anisaldehyde sulphuric acid
Observation: Bluish violet spots indicate the presence of terpenoids.

**In-vitro Anti-Oxidant Activity Using Different Methods**

2.5. Preparation of plant extracts for antioxidant evaluation
About 10 g of the grounded plant were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rounds per
minute for 72 hours at room temperature and stored in refrigerator for 4 days. The extracts were then filtered using filter
papers and concentrated under vacuum on a rotator evaporator. The crude extract was stored at 4 ºC and the antioxidant test
was done directly with in five minutes
2.5. Preparation of plant extracts for antioxidant evaluation
About 10 g of the grounded plant were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rounds per
minute for 72 hours at room temperature and stored in refrigerator for 4 days. The extracts were then filtered using filter
papers and concentrated under vacuum on a rotator evaporator. The crude extract was stored at 4 ºC and the antioxidant test
was done directly with in five minutes
2.5. Preparation of plant extracts for antioxidant evaluation
About 10 g of the grounded plant were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rounds per
minute for 72 hours at room temperature and stored in refrigerator for 4 days. The extracts were then filtered using filter
papers and concentrated under vacuum on a rotator evaporator. The crude extract was stored at 4 ºC and the antioxidant test
was done directly with in five minutes.
2.5. Preparation of plant extracts for antioxidant evaluation
About 10 g of the grounded plant were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rounds per
minute for 72 hours at room temperature and stored in refrigerator for 4 days. The extracts were then filtered using filter
papers and concentrated under vacuum on a rotator evaporator. The crude extract was stored at 4 ºC and the antioxidant test
was done directly with in five minutes.
Antioxidants protects the cell against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

leading to cellular damage. They scavenge free radicals generated during oxidative stress and exhibit a protective effect against many diseases like diabetes, cancer, cardiovascular diseases and ageing. The plants contain a wide variety of free radical scavenging molecules such as flavonoids, phenols, terpenoids and vitamins. The natural antioxidants are ascorbic acid, vitamin E, phenolic acids etc. Natural antioxidants tend to be safer, therefore the evaluation of antioxidant activity of various plant extracts is considered as an important step in the identification of their ability to scavenge the free radicals.

Some of the in vitro models for the evaluation of antioxidant activity are listed below;

- DPPH assay
- Superoxide scavenging assay
- Phosphomolybdenum method

Method 1: Free radical Scavenging activity using diphenyl picryl hydrazyl (DPPH):

The free radical scavenging activity of the extracts is evaluated by assessing their ability to reduce the colour of DPPH in ethanol according to Brand Williams. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts. (Brand Williams et al. 1995, Blios MS 1958)

**Method 2: Determination of scavenging activity against hydrogen peroxide [Ruch RJ et al., 1989]**

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Reagents**

6% hydrogen peroxide diluted with water in the ratio of 1:10

0.1M phosphate buffer pH 7.4

**Method 3:** Total antioxidant activity by Phospho molybdenum Method [Jayanthi P and Lalitha P; 2011]

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Results**

**POWDER MICROSCOPY:**

**ORGANOLEPTIC CHARACTERS**

- NATURE : Coarse powder
- COLOUR : Green
- ODOUR: Odourless.
- TASTE : Palatable taste

Pressed in between two filter paper, No oil mark on the paper.

We have observed the following microscopical cell structures, the powder microscopy of *Onosma bracteatum* results were showed on (Fig-1)

- Epidermis
- Stomata
- Fibers
- Collenchyma
- Xylem vessels
- Phloem

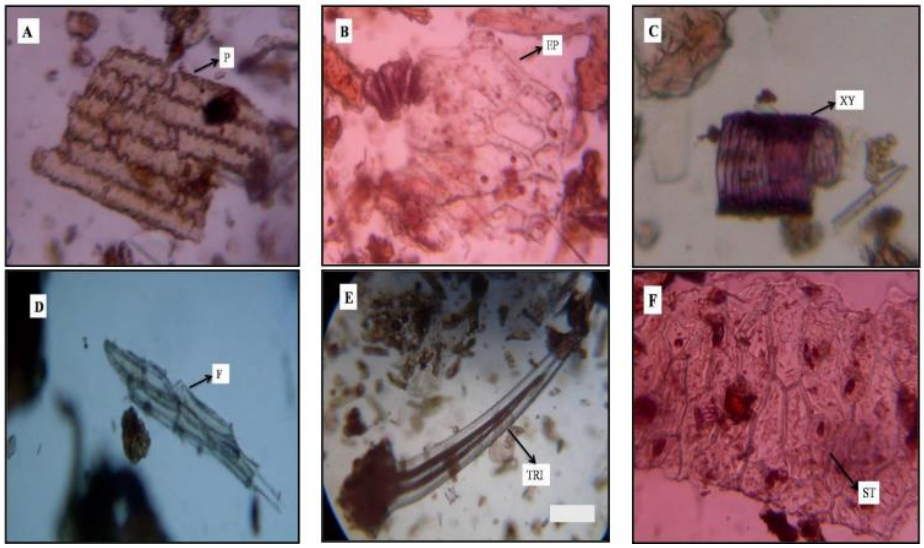



**Figure 1: Powder microscopy of leaf of *Onosma bracteatum*.**

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

**PHYSIOCHEMICAL PARAMETERS**

As per the methods described in materials and methods, physic chemical parameters were carried out and the results were tabulated in (Table-1)

**Table 1: Physiochemical parameters of *Onosma bracteatum* powder**

| Sr. No. | Physiochemical Constant | REPORTS % (w/w) |
|---|---|---|
| **1** | Total ash | 23.17 |
| **2** | Water soluble ash | 4.7 |
| **3** | Acid insoluble ash | 8.9 |
| **4** | SULPHATE ASH | 3.89 |
| **5** | Loss on drying | 0.59 |
| **6** | Foreign Organic Matter | 1.5% |
| **7** | methanol soluble extractive (MSE) value | 23.89 |
| **8** | ethanol soluble extractive (ESE) value | 20.71 |
| **9** | water soluble extractive (WSE) value | 18.09 |
| **10** | Chloroform soluble Extractive (CSE) Value | 6.76 |
| **11** | petroleum ether soluble (PESE) extractive value | 7.81 |
| **12** | ethyl acetate soluble extractive (EASE) value | 8.45 |

The percentage of total ash was found to be 23.17(w/w) and the percentage of water soluble ash was found to be 4.7(w/w) while the acid insoluble ash was 8.9(w/w). The determination of ash values helps to find out where the powdered material was adulterated with sand and other inorganic material. The water-soluble ash helps us to find the amount of inorganic material present in the crude drug, while acid insoluble ash helps us to find the amount of sand and other debris in the crude material.

The percentage of Loss on drying value was found to be 0.59% (w/w) at 105℃ is determined as the presence of excess moisture is Loss conductive to the promotion of mold and bacterial growth, and subsequently to deterioration and spoilage of the drug.

The various extractive values with different solvents have been determined. A maximum extractive value was found with methanol 23.89% (w/w) ethanol 20.71% (w/w) and water 18.09% (w/w). The extractive value helps us to decide what solvent will be useful for extraction of maximum active principle and also helps to decide whether the crude material has already seen exhausted or not. The physiochemical parameters of *Onosma bracteatum* leaves were reported on (Table-1).

**Preliminary Phytochemical Screening of Extract**

The results obtained for the preliminary phytochemical screening of the powder is presented in **Table 2.** From the table, it can observed that the powder showed the presence of phytosterols, proteins, carbohydrates, phenolic compounds, alkaloids, flavonoids, cardiac glycosides. It showed absence for cyanogenetic glycosides, anthroquinone glycosides and purines.

**Table 2: Preliminary Phytochemical Screening of the Powder**

| Sr. No. | Test | methanol | ethanol | water | CHCl3 | Pet Ether | Ethyl Acetate |
|---|---|---|---|---|---|---|---|
| **1** | **Test for Alkaloids** | | | | | | |
| | Mayer's test | + | - | - | + | - | + |
| | Wagner's test | + | - | - | + | - | + |
| | Dragendorff's test | + | - | - | + | - | + |
| **2** | **Glycosides** | | | | | | |
| | Brontrager's Test | - | + | + | + | + | - |
| | Modified Borntrager's Test | - | + | + | + | + | - |

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Keller Kiliani Test | + | + | + | + | + | - |
| | Raymond test | - | + | - | + | + | - |
| | Legal's test | - | + | - | + | + | + |
| **3** | **Test for proteins** | | | | | | |
| | Millon's Test | + | + | + | - | + | + |
| | Biuret Test | + | + | + | - | + | + |
| **4** | **Test for carbohydrates** | | | | | | |
| | Molisch's test | + | + | + | - | + | + |
| | Fehling's test | + | + | + | - | + | + |
| | Benedict's test | + | + | - | - | + | - |
| **5** | Terpenoids | + | + | - | + | + | - |
| **6** | Steroids | + | - | - | - | + | + |
| **7** | Saponins | + | + | + | + | + | + |
| **8** | Tannins | + | + | + | + | + | + |
| **9** | **Phytosterol** | | | | | | |
| | Libermann–buchard's test | + | + | - | + | + | + |
| | Salkowski's reagent | + | + | - | + | + | + |
| **10** | **Flavonoids** | + | + | + | + | + | + |

**Key: '+' – Present, ' - ' – Absent**

The information obtained from preliminary phytochemical screening will be useful in finding out the genuine of the drug. The result of phytochemical screening of powder drug, alcohol, aqueous extract of *Onosma bracteatum* leaf are presented in the phytochemical screening indicated varying quality of alkaloids, carbohydrate, glycoside, sterol, saponins, protein, mucilage, terpenoid, flavonoid, phenol, protein and amino acids in the leaf extract. The phytochemical profile revealed that the methanolic and ethanolic extract contained aldehydes, carbohydrate, glycoside, sterol, saponins, protein, mucilage, tannins, terpenoids and phenols. Aqueous extract contained carbohydrate, glycoside, saponins, mucilage, tannins, terpenoids and phenols. Ethylacetate extract contained aldehydes, carbohydrates, flavonoids, saponins, phenols and tannins. Petroleumether extract contained saponins terpenoids, phenols, glycosides, steroids. Chloroform extract containedaldehydes, steroids, saponins, carbohydrates, flavonoids, phenols, alkaloids, glycosides. Volatile oil and fixed oil were not detected in any of the extract. (Table-2).

**Qualitative Chromatograhic Analysis:**

The results for thin layer chromatography of all the extracts are given in Table 3 and Figure 2. From Table 3, it can be seen that the MSE of *Onosma bracteatum* leaves showed the presence of alkaloids, flavonoids, phytosterols, saponins, terpenoidsand tannins while the ESE showed the presence of cardiac glycosides, flavonoids, phytosterols, saponins, terpenoids and tannins. However, WSE showed the presence of cardiac glycosides, proteins,saponins, tannins and flavonoids while CSE showed the absence of proteins and steroids. PESE showed the presence of all essential components except alkaloids while EASE showed the presence of alkaloids, cardiac glycosides, phytosterols, tannins and terpenes.

**Table 3: Phytochemical screening of extracts of *Onosma bracteatum* Leaves**

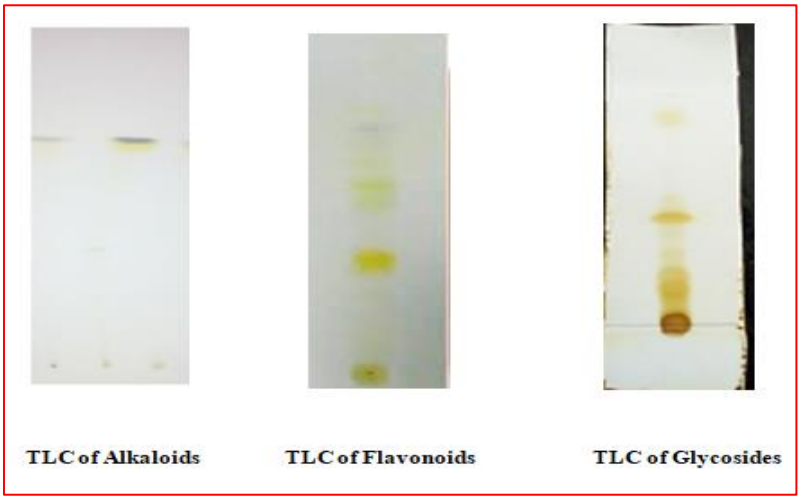
| Extract | Alkaloid | Glycoside | Proteins | Carbohydrates | Terpenoids | Steroids | Saponins | Tannis | Phytosterols |
|---|---|---|---|---|---|---|---|---|---|
| MSE | + | + | + | - | + | + | + | + | + |
| ESE | - | + | + | + | + | - | + | + | + |

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| WSE | - | + | + | + | + | - | - | + | - |
| CSE | + | + | - | - | + | + | + | + | + |
| PESE | - | + | + | + | + | + | + | + | + |
| EASE | + | - | + | + | - | + | + | + | + |
| HAE | + | + | - | + | + | - | - | + | + |



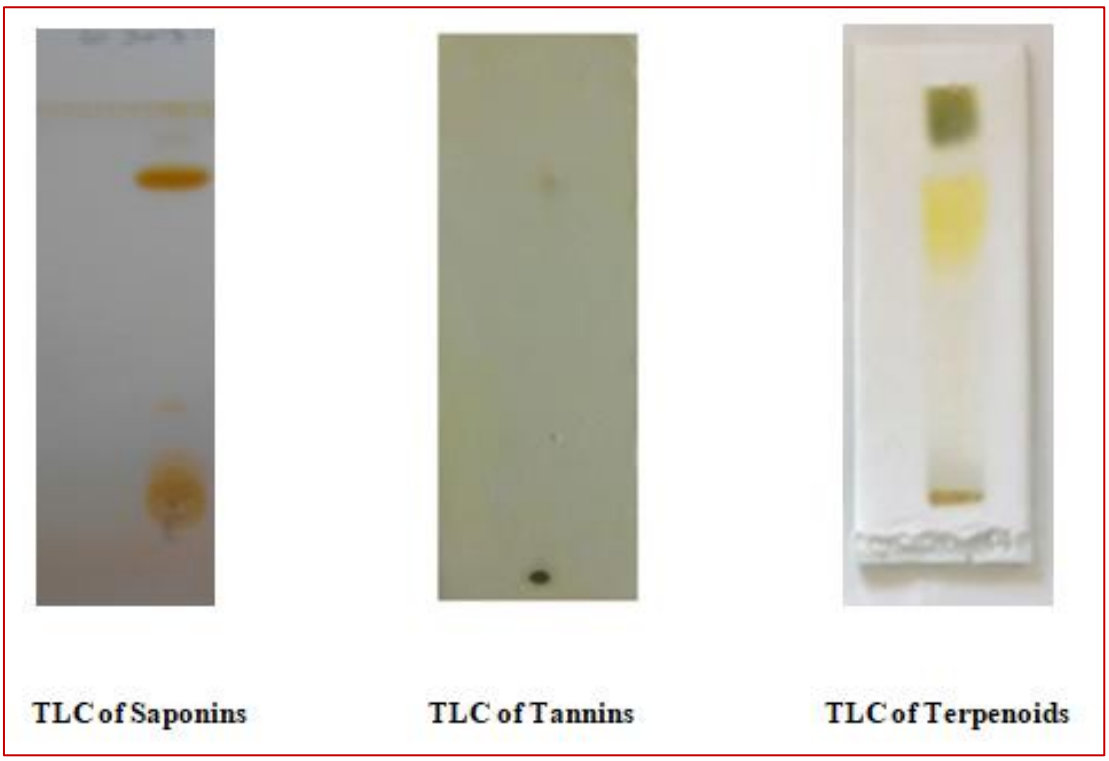




**Figure 2: Thin Layer Chromatography leaves extract**

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

**In-vitro anti-oxidant activity using different methods:**

The in-vitro antioxidant activity of the Methanolic extract of *Onosma bracteatum* leaves was evaluated by five methods. The results obtained for these methods are presented in Tables 15to 19 and the graphical representations are presented in Figs. 30 to 34.

**Method 1: Free radical Scavenging activity using diphenyl picryl hydrazyl (DPPH):**

The results obtained for the free radical scavenging activity against DPPH radical is presented in Table 4.

**Table 4: Percentage inhibition of extract & standard ascorbic acid against DPPH at 517nm**

| **Extract 100 µg/mL** | **Percentage inhibition by extract** | **IC50** µg/mL |
|---|---|---|
| MSE | 85.46 | 21.76 |
| ESE | 64.75 | 33.49 |
| WSE | 24.35 | 89.28 |
| CSE | 58.18 | 43.56 |
| PESE | 61.92 | 38.67 |
| EASE | 76.37 | 28.31 |
| Ascorbic Acid (Control) | 89.36 | 18.21 |

The antioxidant activities of different extracts were indexed by the DPPH radical scavenging activity. As illustrated in Table 4, different extracts possessed varying free radical scavenging activities. Among the extracts tested, methanolic extract was the most potent extract with an $IC_{50}$ value of 21.76 µg/mL with percentage inhibition of 85.46. Compared to methanolic extract, although ethyl acetate and ethanolic extract exhibited a lower radical scavenging activity with respective $IC_{50}$ values of 28.31 µg/mL and 33.49 µg/mL with percentage inhibition of 76.37 and 64.75 respectively. $IC_{50}$ value of methanolic extracts had significantly the same radical scavenging activity as that of ascorbic acid ($IC_{50}$18.21 µg/mL) (Table 4).

**Method 2: Determination of scavenging activity against hydrogen peroxide**

The results obtained for the scavenging activity against hydrogen peroxide arepresented in Table 5.

**Table 4: Percentage Inhibition of Extract & Standard Ascorbic Acid against Hydrogen Peroxide at 230nm**

| **Extract 100 µg/mL** | **Percentage inhibition by extract** | **IC50** µg/mL |
|---|---|---|
| MSE | 81.35 | 18.56 |
| ESE | 58.04 | 31.82 |
| WSE | 22.64 | 84.91 |
| CSE | 53.73 | 39.43 |
| PESE | 55.81 | 32.54 |
| EASE | 72.47 | 25.86 |
| Ascorbic Acid (Control) | 82.48 | 17.24 |

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

The antioxidant activities of different extracts were indexed by the DPPH radical scavenging activity. As illustrated in Table 5, different extracts possessed varying free radical scavenging activities. Among the extracts tested, methanolic extract was the most potent extract with an $IC_{50}$ value of 18.56 µg/mL with percentageinhibition of 81.35. Compared to methanolic extract, although ethyl acetate and ethanolic extract exhibited a lower radical scavenging activity with respective $IC_{50}$ values of 25.86 µg/mL and 31.82 µg/mL with percentage inhibition of 72.47 and 58.04 respectively. $IC_{50}$ value of methanolic extracts had significantly the same radical scavenging activity as that of ascorbic acid ($IC_{50}$17.24 µg/mL) (Table 5).

**Method 3: Total antioxidant activity by Phosphomolybdenum Method**

The results obtained for the scavenging activity against hydrogen peroxide arepresented in Table 6.

**Table 6: Percentage inhibition of extract & standard ascorbic acid Phosphomolybdenum Method at 695nm**

| **Extract 100 µg/mL** | **Percentage inhibition by extract** | **IC50** µg/mL |
|---|---|---|
| MSE | 72.83 | 26.29 |
| ESE | 51.54 | 32.83 |
| WSE | 20.93 | 86.12 |
| CSE | 39.29 | 43.40 |
| PESE | 45.64 | 39.38 |
| EASE | 62.03 | 31.79 |
| Ascorbic Acid (Control) | 75.92 | 23.67 |

The antioxidant activities of different extracts were indexed by the Phosphomolybdenum Method. As illustrated in Table 6, different extracts possessed varying free radical scavenging activities. Among the extracts tested, methanolic extract was the most potent extract with an $IC_{50}$ value of 26.29 µg/mL with percentage inhibition of 72.83. Compared to methanolic extract, although ethyl acetate and ethanolic extract exhibited a lower radical scavenging activity with respective $IC_{50}$ values of 31.79 µg/mL and 32.83 µg/mL with percentageinhibition of62.03 and 51.54 respectively. $IC_{50}$ value of methanolic extracts had significantly the same radical scavenging activity as that of ascorbic acid ($IC_{50}$23.67 µg/mL) (Table 6).

**CONCLUSION:** The result of phytochemical screening of powder drug, alcohol, aqueous extract of *Onosma bracteatum* leaf are presented in the phytochemical screening indicated varying quality of alkaloids, carbohydrate, glycoside, sterol, saponins, protein, mucilage, terpenoid, flavonoid, phenol, protein and amino acids in the leaf extract. The phytochemical profile revealed that the methanolic and ethanolic extract contained aldehydes, carbohydrate, glycoside, sterol, saponins, protein, mucilage, tannins, terpenoids and phenols. The antioxidant activities of different extracts were indexed by the DPPH radical scavenging activity. Different extracts possessed varying free radical scavenging activities. Among the extracts tested, methanolic extract was the most potent extract with an $IC_{50}$ value of 21.76 µg/mL with percentage inhibition of 85.46. Compared to methanolic extract, although ethyl acetate and ethanolic extract exhibited a lower radical scavenging activity with respective $IC_{50}$ values of 28.31 µg/mL and 33.49 µg/mL with percentage inhibition of 76.37 and 64.75 respectively. $IC_{50}$ value of methanolic extracts had significantly the same radical scavenging activity as that of ascorbic acid ($IC_{50}$18.21 µg/mL)

**REFERENCES**

1. Atieno, n. R. (2013). Antibiogram, metal tolerance and plasmid profiles of pathogens isolated from wastewaters and sludge of abattoirs in nairobi, kenya (doctoral dissertation, kenyatta university).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 2011-2022 | ISSN:2251-6727

2. Bele, A. A., & Khale, A. (2011). An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research*, *2*(2), 256.
3. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.
4. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.
5. Cipriano, R. C., & Bullock, G. L. (2001). *Furunculosis and other diseases caused by Aeromonas salmonicida*. Charles Town, WV: National Fish Health Research Laboratory.
6. Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences.
7. Finar, I. L., & Finar, I. L. (1967). *Organic chemistry: the fundamental principles* (Vol. 1, p. 434). London: Longmans.
8. Gasimova, A., Mammadov, R., Tatlı, B., & Alper, M. (2024). Determination of radical scavenging activity, secondary metabolite amount, and toxicity of Onopordum bracteatum extracts. *Sigma Journal of Engineering and Natural Sciences*, *42*(5), 1612-1620.
9. Gokhale, M. S., & Kokate, C. K. (2008). *Practical pharmacognosy*. Editora Record.
10. Harborne, J. B., & Harborne, J. B. (1973). Phenolic compounds. *Phytochemical methods: A guide to modern techniques of plant analysis*, 33-88.
11. Hassan, M. E., Shehata, H. A., Fahmy, A., Badr, M., Tamer, T. M., & Omer, A. M. (2021). Development of biodegradable poly (vinyl alcohol)/chitosan cross linked membranes for antibacterial wound dressing applications. *Jordan Journal of Biological Sciences*, *14*(1).
12. Jayanthi, P., Lalitha, P., & Aarthi, N. (2012). Larvicidal and pupicidal activity of extracts and fractionates of Eichhornia crassipes (Mart.) Solms against the filarial vector Culex quinquefasciatus Say. *Parasitology research*, *111*, 2129-2135.
13. Kennedy, B. M., Kharaka, Y. K., Evans, W. C., Ellwood, A., DePaolo, D. J., Thordsen, J., ... & Mariner, R. H. (1997). Mantle fluids in the San Andreas fault system, California. *Science*, *278*(5341), 1278-1281.
14. Kokate, T. G., Cohen, A. L., Karp, E., & Rogawski, M. A. (1996). Neuroactive steroids protect against pilocarpine-and kainic acid-induced limbic seizures and status epilepticus in mice. *Neuropharmacology*, *35*(8), 1049-1056.
15. Lachance, H., Wetzel, S., Kumar, K., & Waldmann, H. (2012). Charting, navigating, and populating natural product chemical space for drug discovery. *Journal of medicinal chemistry*, *55*(13), 5989-6001.
16. Nicolaides, A. N., Harbourne, T., & Russell, R. C. G. (1984). Subcutaneous heparin, plasma heparin levels and postoperative wound haematoma. *British journal of surgery*, *71*(1), 62-64.
17. Parekh, P., Dutkiewicz, S., Follows, M. J., & Ito, T. (2006). Atmospheric carbon dioxide in a less dusty world. *Geophysical research letters*, *33*(3).
18. Pattanayak, S. (2019). Healthcare system using succulent parts of plants. *Volume*, *1*, 01-144.
19. Rajapara, A. M., Bhatt, V. P., & Shah, M. B. (2021). Phytochemical and pharmacological investigation on Onosma bracteatum Wall. *Asian J Biol Life Sci*, *10*, 141-149.
20. Rhyu, M. R., Lu, J., Webster, D. E., Fabricant, D. S., Farnsworth, N. R., & Wang, Z. J. (2006). Black cohosh (Actaea racemosa, Cimicifuga racemosa) behaves as a mixed competitive ligand and partial agonist at the human μ opiate receptor. *Journal of agricultural and food chemistry*, *54*(26), 9852-9857.
21. Siddiqui, Z. A., & Mahmood, I. (1995). Some observations on the management of the wilt disease complex of pigeonpea by treatment with a vesicular arbuscular fungus and biocontrol agents for nematodes. *Bioresource Technology*, *54*(3), 227-230.
22. World Health Organization. (1998). *Quality control methods for medicinal plant materials*. World Health Organization.