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Comparative Study on The Phytochemical, Antistaphylococcal and Antioxidant Properties of the Stem Bark of *Jatrophacurcas* L and *Jatrophagossypifolia* L

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ABSTRACT: This study was conducted to assess the phytochemical, antistaphylococcal and antioxidant properties of the leaf and stem bark of *Jatrophacurcas* and *Jatrophagossypifolia*. Ethanolic extracts of the leaf and stem bark of *J. curcas* and *J. gossypifolia* were obtained using standard methods. Qualitative and quantitative phytochemical properties of the Jatropha plant was assessed using standard methods. Antistaphylococcal and minimum inhibitory concentrations of the extracts were assessed against *Staphylococcal aureus* obtained from different sources using agar diffusion method. The ferrous ion and hydroxyl radical scavenging activity of the extracts were also determined using standard methods. Quantitative phytochemical screening revealed that the distribution of phytochemicals in the two *Jatropha* species do not follow a regular trend. However, saponin was highest in *J. curcas* stem bark (35.64 mg/g) while *J. gossypifolia*stem bark and leaf have the highest flavonoid (31.35 mg/g) and alkaloids (23.20 mg/g) respectively. The antistaphylococcal effect of the combined extract was higher and significantly different ($P \leq 0.05$) than when used singly. The highest antistaphylococcal effect (19.83mm) was recorded for the combination of *J. curcas* and *J gossypifolia* leaf (JCL and JGL) against *Staphylococcus aureus* obtained from blood. Antioxidant assay of extracts revealed a concentration-dependent effect. The antioxidant activities of the extracts vary from one extract to the other. The results obtained from this study indicates that bioactive compounds present in *J. curcas* and *J gossypifolia* can be exploited as source of effective antistaphylococcal and antioxidant compounds.

Keywords: Comparative; Antioxidant; Antistaphylococcal; Leaf; Stem bark; Extract; *Jatrophacurcas*; *Jatrophagossypifolia*.

دراسة مقارنة حول الخصائص الكيميائية النباتية والمضادة للمكورات العنقودية ومضادات الأكسدة للحاء الجزعي لنباتي من صنف الجاتروفاكوركاس وجاتروفا جوسييفوليا

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الملخص:أجريت هذه الدراسة لتقييم الخصائص الكيميائية النباتية والمضادة للمكورات العنقودية والمضادة للأكسدة من أوراق وسيقان الجاتروفا كوركاس و جاتروفا جوسييفوليا. تم الحصول على المستخلص الكحولي من أوراق وسيقان الجاتروفا كوركاس و جاتروفا جوسييفوليا باستخدام الطرق القياسية. تم تقييم الخصائص النوعية والكمية الكيميائية النباتية لنبات الجاتروفا باستخدام الطرق القياسية. تم تقييم النشاط المضاد الميكروبي للمكورات العنقودية الذهبية التي تم الحصول عليها من مصادر مختلفة باستخدام طريقة انتشار الأجار. تم تحديد الأنزيمات المضادة للأكسدةو الشوارد الحرة للمستخلصات أيضًا باستخدام الطرق القياسية. كشف الفحص الكيميائي النباتي الكمي أن توزيع المواد الكيميائية النباتية في نوعي جاتروفا تختلف من نوع لأخر. ومع ذلك ، كان السابونين أعلى في لحاء جذع جاتروفا كركاس (35.64 مجم/جم) ، بينما كان لحاء ساق الجاتروفا جوسيفوليا وأوراقه أعلى نسبة من الفلافونويد (31.35 مجم/جم) والقلويدات (23.20 مجم/جم) على التوالي. كان التأثير المضاد للمكورات العنقودية لكلا المستخلصين معا أعلى ومختلفًا اختلافًا معنويًا ($P \leq 0.05$) عند استخدامه منفردًا. تم تسجيل أعلى تأثير مضاد للمكورات العنقودية (19.83 مم) لمزيج من الجاتروفا كركاس وأوراق الجاتروفا جوسيفولياضد المكورات العنقودية الذهبية المأخوذة من الدم. وتبين من نتائج مضادات الأكسدة للمستخلصات انها تتناسب طرديا مع التركيزات. تختلف الأنشطة المضادة للأكسدة في المستخلصات من مستخلص إلى آخر. تشير النتائج التي تم الحصول عليها من هذه الدراسة إلى أن المركبات النشطة بيولوجيًا المستخلصة من أوراق وسيقان الجاتروفا كوركاس و جاتروفا جوسييفوليا و يمكن استغلالها كمصدر فعال لمضادات المكورات العنقودية ومضادات الأكسدة.

الكلمات المفتاحية: مقارنة؛ مضادات الأكسدة، مضادات المكورات العنقودية ؛ اللحاء الجذعي ؛ جاتروفا كركاس جاتروفا جوسييفوليا.

1. Introduction

Diseases of man caused by microorganisms have become a major threat to human health and existence. *Staphylococcus* species are very important pathogen responsible for bacterial infections in hospitals and communities worldwide. They are very diverse and are implicated in various infection processes especially in immune-compromised individuals and those with implant devices such as shunts and catheters [1]. *Staphylococcus aureus* has been recognized as a versatile microorganism worldwide [2]. It is a human pathogen and a part of the normal flora of human skin [3]. It can colonize and infect both patients and healthy people with life threatening effects [4]. *Staphylococcus*species generally exhibit multiple antibiotic resistances [5, 6].

Resistance of microorganisms to commonly used antimicrobial agents is a major challenge in the treatment of diseases of microbial origin. Hence, the search for antimicrobial agents that are safe and most importantly effective against diseases of microbial origins has doubled/tripled in the last three decades. This is as a result of resistance developed by microorganisms against commonly used antimicrobial agents, the safety of these antimicrobial agents and the side effects associated with the use of antibiotics in the treatment of gastroenteritis and other infections [7].

Plant-based bioactive compounds have recently become of great interest in the search for suitable, safe and friendly alternatives to those existing antimicrobials which are becoming less effective. Researchers have made tremendous efforts to discover new antimicrobial compounds from natural products especially of plant origin. Ncube *et al*. [8] submitted that medicinal plants are the richest bio-resource of drugs of the traditional system of medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Moreover, many people all over the world have resorted to the use of herbal medicine for the treatment of various health challenges [9].

Jatropha species are among these medicinal plants that have attracted interest as potential sources of antimicrobial agent. They belong to the Family Euphorbiaceae. They contain secondary metabolites such as alkaloids, tannins, flavonoids, steroid, saponins and phenolics which are known to possess medicinal properties [10, 11]. The stem bark, root, leaf, sap, seed and the oil from the seed of *Jatropha* species had been used for treating various ailments ranging from skin diseases, parasitic diseases, urinary tract infections, bleeding of gum and toothaches, treatment of wound and sores, fever and many others [12-18].

Jatropha curcas and *Jatropha gossypifolia* are commonly found in Nigeria. These two plants had found wide use in ethno-medicine especially in rural parts of Nigeria. The present study is therefore aimed at comparative study of the phytochemical properties, antioxidant and antistaphyloccal activities of leaf and stem bark extracts of *J. curcas* and *J. gossypifolia*singly and in combination.

2. Materials and methods

2.1 Collection and preparation of *Jatropha* species extracts

Fresh leaves and stem bark of *Jatropha curcas* L and *Jatropha gossypifolia* L were collected from a local farm at Oke-Aro in Akure, Ondo State, Nigeria. The plants were identified and authenticated by a plant scientist in the Department of Crop Science and Pest Management, Federal University of Technology, Akure. The stem bark and leaf were rinsed with clean water and sun-dried for three weeks under shade and then pulverized using a mechanical grinder. The pulverized plant material was kept in an air-tight cellophane bag until required. Powdered *Jatropha* species (200 g) of the leaf and stem bark were each placed in 1000 ml of 99.7% ethanol (analytical grade) and kept in conical flasks, each was shaken in a rotary shaker at 121rpm for 24 hrs. After 24 hours, the suspension was filtered with a double-layer muslin cloth and Whatman No. 1 filter paper. The resulting filtrates were concentrated under reduced pressure in a rotary evaporator (RE - 52A; Union Laboratory, England) at 40 ^{o}C.

2.2 Test organism,*Staphylococcus* species

All *Staphylococcus* species were grown on Mannitol salt agar plates at 37 ^{o}C for 24 hours. The isolates were maintained on agar slant and stored in the refrigerator at 5 ^{o}C until used.

2.3 Quantitative phytochemical analysis of *Jatropha* species

Quantitative phytochemical screening of the crude extracts of the stem bark and leaf of *Jatropha* species was performed using standard procedures as described by Harborne [19]; Trease and Evans [20]. The quantity of the following phytochemicals viz; saponin, tannin, flavonoid, cardiac glycosides and alkaloids were assessed in the extracts obtained from the two *Jatropha* species.

2.4 Determination of total phenolic content

The total phenol content of leaf and stem bark was determined (Gallic acid equivalent) as described by Singleton *et al*. [21] with slight modifications. Briefly, 200µL of the extract dissolved in 10% DMSO (240 μgmL^{-1}) was incubated with 1.0 ml of Folin-Ciocalteu reagent (diluted 10 times) and 800 µL of 0.7 mol L^{-1} Na_2CO_3 for 30 minutes at room temperature. Then, the absorbance was measured at 765 nm on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu, Japan). All measurements were done in triplicates. Results are expressed as mg GAE / 100 g dry ethanol extracts.

2.5 Determination of antistaphylococcalproperties of *Jatropha* species

Antistaphylococcal activity of stem bark and leafextracts of *Jatropha* species was determined by agar well diffusion method as described by Abubakar*et al*. [22]. *Staphylococcus* species obtained from different sources were cultivated on nutrient broth at 32 °C for 18 hours. The inoculum size was adjusted by serial dilution to obtain 0.5 McFarland turbidity standards. The extract was reconstituted in 20% v/v of Dimethyl sulfoxide (DMSO). An aliquot of 0.1 mL containing organism was aseptically transferred and evenly spread onto the dried surface of the sterile Mueller Hinton agar plate. A well of 8 mm was bored in the agar plate with a sterile cork borer. Each extract was sterilized through a membrane filter (0.22 µm) and 0.1 mL was aseptically introduced into the well in the Petri dishes already inoculated with *Staphylococcus* species with the aid of a micropipette. A volume of 0.1 mL of Ciprofloxacin was used as positive control while 20% of DMSO served as a negative control. The plates were incubated at 37 °C for 24 hours. The diameter of the inhibition zones was measured in millimeters.

2.6 Antioxidant assay

The following antioxidant assays were performed on the stem bark and leaf extracts obtained from *Jatropha* species.

2.7 Hydroxyl radical scavenging ability of Stem Bark and Leaf extracts of *Jatropha* species

The determination of the scavenging effect on hydroxyl radicals was carried out as described by Oyetayo *et al.* [23]. The reaction mixtures in a final volume of 1.0 ml, containing 0.4 ml of 20 mmol/ml sodium phosphate buffer (pH 7.4), 0.1 ml of 0.125-2 mg/ml extracts, 0.1 ml of 60 nmol/L deoxyribose, 0.1 ml of 10 mmol/L hydrogen peroxide, 0.1 ml of 1 mmol/L ferric chloride, 0.1 ml of 1.04 mmol/L EDTA and 0.1 ml of 2 mmol/L ascorbic acid was incubated at 37 ℃ for 1 hour. Solutions of $FeCl_2$ and ascorbic acid were made up immediately before use in de-ionized water. The reaction was stopped by adding 1 ml of 17 mmol/L thiobarbituricacid (TBA) and 1 ml of 17 mmol/L trichloroacetic acid (TCA). The mixture was boiled for 15 min, cooled in ice and then the absorbance was measured at 532 nm using a UNICO 2100 spectrophotometer (As).

2.8 Ferrous ion chelating ability assay

The Fe^{2+}chelating ability of leaf and stem bark ethanol extracts was determined by employing a modified method of Puntel *et al*. [24]. Freshly prepared 500 µmol L^{-1} $FeSO_4$ was added to a solution containing 168µL of 0.1 $molL^{-1}$Tris-HCl (pH 7.4), together with 218 µL of saline and an ethanol extract (1-5 mg /ml). The solution was incubated for 5 minutes, followed by the addition of 13 µL of 0.25%, 1,10 phenantroline (w/v). Absorbance was read at 510 nm. Fe^{2+}chelating ability was expressed as percentage inhibition.

2.9 Statistical analysis

Experiments were carried out in replicates and data obtained were analyzed by one way analysis of variance (ANOVA) and means were separated by Duncan multiple range test (SPSS 17.0 version). Differences were considered significant at $P \leq 0.05$.

3. Results

The distribution of the phytochemicals in the two *Jatropha* species does not follow a regular trend as revealed by quantitative phytochemical screening (Table 1). Saponin was highest and significantly different (P≤0.05) in *J. curcas* stem bark (35.64 mg/100 g) when compared to other extracts, while *J. gossypifolia* stem bark and leaf has the highest flavonoid (31.35 mg/100 g) and alkaloid (23.20 mg/100g) respectively. The phenolic contents of the different parts of the *Jatropha* species vary and are significantly different (P≤0.05). The leaf extract of *J. curcas* has the highest total phenol (2.2 mgGAE/g) while the least was recorded in the leaf extract of *J. gossypifolia* (0.8 mgGAE/g) (Figure 1).

Table 1. Quantitative Phytochemical Contents of Leaf and Stem Bark Extracts of Two *Jatropha* Species.

Phytochemicals	JCL	JCS	JGL	JGS
Saponin	4.30 ± 0.29^{a}	35.64 ± 1.56^{c}	14.03 ± 1.21^{b}	13.67 ± 0.41^{b}
Tannin	0.93 ± 0.03^{a}	0.98 ± 0.12^{a}	7.20 ± 0.93^{b}	9.02 ± 0.23^{c}
Flavonoid	1.11 ± 0.05^{a}	10.15 ± 0.28^{b}	22.81 ± 0.37^{c}	31.35 ± 2.38^{d}
Cardiac glycosides	7.73 ± 0.32^{d}	1.95 ± 0.06^{a}	5.15 ± 0.14^{b}	6.85 ± 0.23^{c}
Alkaloid	3.87 ± 0.11^{a}	5.05 ± 0.06^{a}	23.20 ± 2.42^{b}	2.90 ± 0.11^{a}

Key
JCL: *J. curcas* leaf extract, JCS: *J. curcas* stem bark extract.
JGL: *J.gossypifolia* leaf extract, JGS: *J. gossypifolia* stem bark extract.

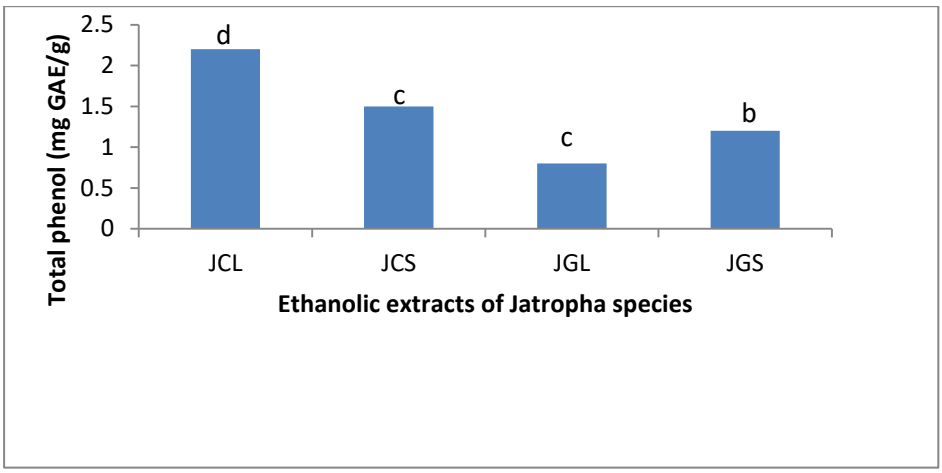

Figure1. Total Phenolics of extracts obtained from Jatropha species.

Bar with different superscript are significantly different (P≤0.05)
Key
JCL: *J. curcas* leaf extract
JCS: *J. curcas* stem bark extract
JGL: *J.gossypifolia* leaf extract
JGS: *J. gossypifolia* stem bark extract

There were significant differences between and within groups in the antistaphylococcal activity of the extracts against Staphylococci used in this study except for *J. curcas* leaf extract (JCL) and *J. gossypifolia* stem bark extract (JGS) where there were no significant differences (P≥0.05) within the two groups (Table 2). The antistaphylococcal activity of the combined extracts was higher and significantly different (P≤0.05) than when used singly. The highest antistaphylococcal effect (19.83 mm) was obtained for the combination of the leaf extracts of the two *Jatropha* species (JCL and JGL) against *S. aureus* isolated from blood. *J.curcas* stem bark extract (JCS) displayed the least antistaphylococcal effect of 7.00mm against *S.aureus* isolated from urine.

Table 2. Antistaphylococcal Property of the Stem Bark and Leaf extract of *Jatropha* species.

Source of Staph.	JGL	JCL	JGS	JCS	JCL&JGL	JCS&JGS	CPX
POW	10.50±1.73	15.25±0.96	16.25±2.22	9.50±4.04	15.75±2.63	18.25±0.50	21.25±2.22
Skin Swab	11.00±1.93	13.13±3.36	14.00±3.42	11.50±4.31	17.75±4.03	14.00±1.07	24.25±1.67
Urine	12.00±1.43	11.80±4.66	11.60±4.72	7.00±1.00	15.00±1.22	14.20±1.10	32.60±1.14
Blood	12.50±0.55	13.67±1.03	15.00±2.19	12.17±2.64	19.83±2.99	16.33±1.37	22.83±1.17
Nose	10.93±1.81	14.62±2.45	14.65±3.16	12.46±3.16	15.04±1.93	14.92±2.17	24.23±1.75
ATCC 25923	14.33±0.58	12.67±0.58	12.33±1.53	7.33±1.53	17.00±1.00	13.83±0.76	27.33±1.16

Key
POW: Post Operative Wound
JCL: *J. curcas* leaf extract
JCS: *J. curcas* stem bark extract
JGL: *J.gossypifolia* leaf extract
JGS: *J. gossypifolia* stem bark extract
CPX: Ciprofloxacin

The antioxidant capacity of the extracts is revealed in Figures 2 and 3. Ferric ion chelating activity of the two *Jatropha* species was observed to be concentration dependent. The higher ferric ion chelating effect (72%) was displayed by extract obtained from the stem bark of *J. curcas* (JCS) while the least (56%) was recorded for extract obtained from *J. gossypifolia* stem bark extract (JGS) at a concentration of 5 mg/mL. Hydroxyl ion scavenging activities of the *Jatropha* species were also concentration dependent. The highest hydroxyl ion scavenging ability (88%) was obtained for *J. curcas* lea extract (JCL) while the least (55%) was obtained for *J. gossypifolia* leaf extract (JGL).

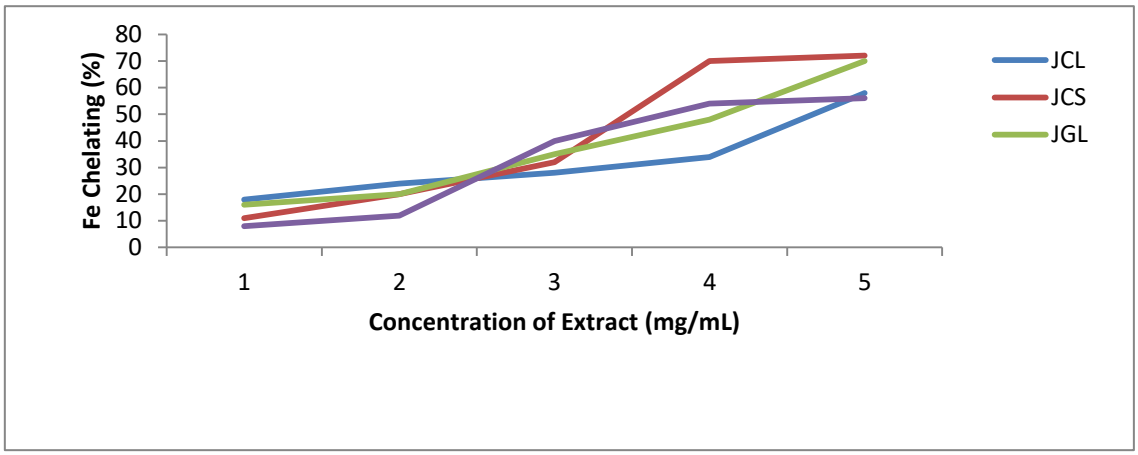

Figure 2. Ferric ion Chelating Ability of of the Stem Bark and Leaf Extracts of Two *Jatropha* species.

Key
JCL: *J. curcas* leaf extract
JCS: *J. curcas* stem bark extract
JGL: *J.gossypifolia* leaf extract
JGS: *J. gossypifolia* stem bark extract

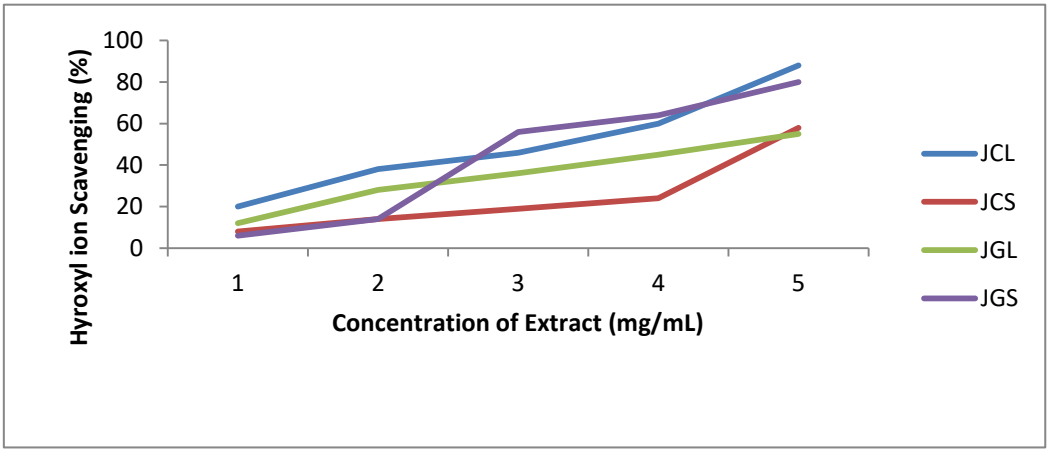

Figure 3. Hydroxyl ion Scavenging of Leaf and Stem Bark Extracts of Two *Jatropha* Species.

Key
JCL: *J.curcas* leaf extract
JCS: *J.curcas* stem bark extract
JGL: *J.gossypifolia* leaf extract
JGS:*J.gossypifolia*stem bark extract

4. Discussion

The medicinal properties of plants have been identified since time immemorial and they are now recognized as a source of bioactive compounds which can be exploited for the development of novel biopharmaceutical agents. Plants synthesize secondary metabolites, many of which are important in promoting good health in animals and humans [25]. These secondary metabolites such as alkaloids, tannins, anthraquinones, saponin, glycosides and so on are important for the survival of plants in their ecosystem. *Jatropha* species are important medicinal plants that have folkloric applications.

In the present report, quantitative phytochemical screening of ethanolic extracts obtained from the leaf and stem bark of two *Jatropha* species, *J. curcas* and *J. gossypifolia*, was not similar to the report of Atamgba *et al*. [26] which revealed that the phytochemicals are more present in leaf than in the stem bark. However, in the present report, saponin and flavonoid were higher in *J. curcas* stem bark (35.64 mg/100 g) and *J. gossypifolia* stem bark extract (31.35

mg/100g) while cardiac glycosides and alkaloids were highest in the leaves of *J. curcas* (7.73 mg/100 g) and *J. gossypifolia* (23.20 mg/100 g) respectively. There was no relative increase in the phytochemical content as one moved from the stem bark to the leaf. Secondary metabolites such as alkaloids, tannins, anthraquinones, saponin, and glycosides produced by plants have potent antimicrobial effects [27]. These secondary metabolites are known to exert considerable antimicrobial activity through different mechanisms [28]. Specifically, saponins are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by enzymes in response to pathogen attacks [29, 30]

The total phenol content obtained in the leaf and stem bark of the two *Jatropha* species varies from 0.8 mgGAE/g to 2.2 mgGAE/g. Othman *et al*. [31] had earlier reported the total phenol content in leaves and stem of *J. curcas* plant collected from Malaysia as 1.33 mg GAE/g and 0.11 mg GAE/g respectively. Akhtar *et al*. [32] also reported higher level of total phenol in the leaf of *J. curcas* than the stem bark as observed in this study. The total phenol content in the leaf (2.2 mgGAE/g) and stem bark (1.5 mgGAE/g) of *J. curcas* were however higher and significantly different from what was obtained in *J. gossypifolia* leaf and stem bark. *J. curcas* had been reported to have high content of phenolic compounds [33, 34]. Recently, Vega-Ruiz*et al*. [35] also reported that *J. cinerea* and *J. cordata* two species of *Jatropha* collected in Mexico are important source of phenolic acids and flavonoids.

The ethanolic extracts of the two *Jatropha* species show good antistaphylococcal effects. There was no significant difference in the antistaphylococcal activities exhibited by the leaf and stem extracts of the plant. However, combination of the leaf extracts and stem bark extracts of the two *Jatropha* species exhibited higher and significantly higher antistaphylococcal effect. Rampadarath *et al*. [36] reported inhibition of the growth of *S. aureus, S. epidermidis, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, B. subtilis* and *Proteus vulgaris* by methanolic extract of the leaves of *J. curcas*. Moreover, Igbinosa [37] had earlier observed the antibacterial effect of the crude root extracts of *J. carcus*. In another report, the essential oil obtained from J. gossypifolia was found to exhibit strong antibacterial activity against *Escherichia coli, Enterococcus faecium,* and *S. aureus* [38]. The antibacterial effect observed in this study may lay credence to the ethnomedicinal uses of *Jatropha* species in the treatment of wounds and sore. Felger and Moser in 1973 [35] reported the use of *J. cinerea* roots by the Seri ethnic group in the state of Sonora (Mexico) to cure dysentery and the sap to treat mouth ulcers. Another species of *Jatropha, J. cordata* root is also used by ethnic groups in the state of Sonora, Mexico to combat toothache and the stem and leaves are used to cure gum disease [39].

Extracts obtained from the leaf and stem bark of *J. curcas* and *J. gossypifolia* exhibited concentration-dependent antioxidant activity. Sunday *et al.* [40] had earlier observed that extracts of *J.curcas*(Leaves and Stem bark extract) and *J. gossypifolia* (Leaves and Stem bark extract) exhibited effective antiradicals' potencies against the different oxidants, indicating they are good electron donors. In another recent report, extracts of leaves and stems from *J. cinerea* and *J. cordata* collected in Mexico were found to possess good antioxidant activity [35]. The antioxidant activities of plants had been attributed to the total phenol and other antioxidant compounds such as volatile oils, amino acids, vitamins and others [41]. The total phenol obtained in this study was high enough to elicit appreciable antioxidant activity. It has been reported that a significant correlation between antioxidant activity and total phenolic contents is prove that phenolic compounds is a major contributor to the antioxidant activity of different parts of *Jatropha*s pecies [32].

5. Conclusion

The results obtained from this study conclusively indicate that extracts obtained from *J. curcas* and *J. gossypifolia* possess effective antistaphylococcal and antioxidant properties. There was no much difference in the antistaphylococcal and antioxidant properties of ethanolic extracts obtained from the two *Jatropha* species; however, the combination of the extracts produced the better antistaphylococcal effect. The bioactive compounds present in the two *Jatropha* species may therefore be exploited as a source of effective antistaphylococcal and antioxidant compounds.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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