



Phytochemical Study and Antimicrobial Activity of Two Medicinal plants from Al-Baha region of Saudi Arabia

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ABSTRACT

The plant is a huge therapeutic source with enormous applications in curative industry. For new sources of antimicrobial agents, chloroform, ethyl acetate, and n-butanol extracts of two medicinal plants (*Dodonaea viscosa* and *Capparis spinosa*) were prepared by liquid-liquid extraction. The plants were collected from Shuda mountain/Al-Baha region/Saudi Arabia and then evaluated systematically. Phytochemical screening tests detect the existence of flavonoids, alkaloids, tannin, terpenoids, saponins and carbohydrates in most of the performed extracts. Antimicrobial activity was assessed against five bacterial and one fungal race. The extracts of the two plants leaves showed wide vision antibacterial activity and significant antifungal activity. The antibacterial activity (zone of inhibition) of *Dodonaea viscosa* species varied from 30 to 18 mm, whereas of *Capparis spinosa* the range from 20 to 16 mm for ethyl acetate extracts, for n-butanol extracts the inhibition zone differ from 15–10 and 16-10 mm respectively. These results indicated that antimicrobial activities of plant species differ to a wide range not only between species themselves but also within the tests for antimicrobial evaluation. The current result supports the medicinal use of the leaves of these studied plants which works as an antimicrobial agent. These results compare to other studies carried out for the same plants in different countries in different environments exhibited diverse outcome in chemical constituents in the plant extracts and in their effects on tested types of micro organisms which may have been due to a number of factors, including geographical location, season and environmental factors, and the method of extraction. This study for the two plant species was first time performed in this area of Saudi Arabia.

Keywords: *Capparis spinosa*, *Dodonaea viscosa*, Antimicrobial activity, Phytochemical screening, Zone of inhibition, Minimum inhibition concentration.

INTRODUCTION

Medicinal plants contain most ingredients in local medicines and most of Western prepared medicines comprise one or additional ingredients of plant origin¹. Medicinal plants were used in folk medicine in various civilizations since ancient times

due to its strong therapeutic effect on humans². Medicines extracted from plants remain important sources, particularly in developing countries, to fight serious diseases. Approximately 60 to 80% of humans in the world still rely on folk remedies for the curing of popular diseases³. It is preferable to use manufactured medicines for several decades but



now prefer to use herbal medicines to prevent sightless dependence on synthetic drugs. Latterly, plentiful plants have been assayed and notified for their power of the propagation of microorganisms, which can substituted the chemically manufactured medicines².

Dodonaea viscosa (Sapindaceae) is considered as evergreen local plants in Saudi Arabia and it is spread in most warm countries⁴. *Dodonaea viscosa* has several therapeutic properties and is used as a folk remedy in many countries, it is given orally or as scumble to remedy many diseases⁴. In variety African and Asian countries, people prepared the yield of boiled dried leaves for curing of stomach ulcer after grinding and mixing them with milk or honey, and is involved in the therapy of hemorrhoids and stomach pain concerning to the liver and spleen⁴. Moreover to their traditional uses, several surveys have proved their therapeutic value, as they confirm their anti-inflammatory effect, anti-oxidation and hypolipidaemic effect⁴.

Capparis species pertinence to family capparaceae is not an exception to therapeutic properties. This family comprise of 250 species, including *C. spinosa* and *C. decidua* are domestic to Saudi Arabia which has been reported for their uses in traditional medicine in humans (Mabberley, 1997; Hamed *et al.*, 2007). Upper parts of these sorts have been notified to be effective against bacteria. The extract of the fermented plant parts have been conveyed to be effective to preventing the evaluation of diverse bacterial strains, especially those that show resistance to medications such as ciprofloxacin, vancomycin and teicoplanin².

The studies in the field of medicines exhibit that *Dodonaea viscosa* hold over antidiabetic, antimicrobial, insecticidal, antioxidant, cytotoxic, antifertility, anti-inflammatory, analgesic, anti-ulcer, antispasmodic, anti-diarrheal and detoxification influence⁵.

Scientifically, the phytochemical researches of capers extracts offered the presence of numerous chemical classes with very interesting biological activities. Ingredients present are alkaloids, fatty acids, phenolic acids, flavonoids, aldehydes, esters, vitamins and glucosinolates. Other studies by Ali-Shtayeh & Abu-Ghdeib (1999) established that the water extract of *Capparis spinosa* has antifungal

characteristics together with a percentage of suppression greater than 90% against *T. violaceum* which in charge of ringworm and which is wide difuce in Africa and Asia⁶.

The targets of this investigation were to evaluate the role of ethanol, chloroform, ethyl acetate, and n-butanol extracts of leaves of *D. viscosa* and *C. spinosa* from Jebal Shuda/Al-Baha area for potential antimicrobial effect, against human infective bacteria, yeast (*Candida albicans*), and plant infective bacteria, and also, study the (MIC), and (MBC) of the plant extracts, and to trial the sensitivity of infective microorganisms for antibiotics, and compared it with the effect of the plant extracts.

MATERIALS AND METHODS

Material

The plant samples leaves (*Dodonaea viscosa* and *Capparis spinosa*) were gathered from Shuda mountain in Almahwah region on march 2018 were classified, confirmed by Biology department, a voucher samples were kept in Biology department College of Sciences and Arts/Almahwah, Al-Baha University.

Preparation of the ethanol extract

The leaves samples of the two plants were cleaned dried in shade for one month at room temperature and powdered. The powdered materials (500 g) were macerated in 3 liters of 80% ethanol and left for three days at room temperature. The samples then filtered and the collected filtrates were concentrated with rotary evaporator, after that the extracts were dried at room temperature for 10 days. Eventually the extracts were kept in vacuum desiccators for three days for complete dryness.

Fractionation of the ethyl alcohol extract through liquid – liquid extraction

Each ethyl alcohol extract was dissolved in 200 mL of distilled water and then was transferred to separatory funnel, subsequently it was extracted consecutively with the organic solvents in order of increasing polarities initiated with petroleum ether, then chloroform, ethyl acetate and n-butanol. The organic layers were separated and concentrated, with rotary evaporator and then drain off at room temperature for ten days.

Screening of Phytochemical Components

The leaves extracts were subjected to specific tests using the standard methods for the establishment of the chemical components⁷⁻⁸.

Test for Phenolics

The extract (0.25 g) was dissolved in 10 mL of water and then refined. A small drop of 0.1% ferric chloride was added to the filtrate. The occurrence of strong brownish-green or a blue-black color represents the existence of phenolics in the samples.

Test of tannins

The dried ground sample (0.5 g) was boiled in 15 mL distilled water in small beaker and filtered. A little quantity of 0.1% ferric chloride was added. The occurrence of brownish green or blue-black colour proven the existence of tannin in the sample.

Test of steroids

Acetic anhydride (2 mL) was added to 0.5 g ethanolic extract of each specimen with 2 mL sulphuric acid. Changes of the tint from purple to blue or green represent the occurrence of steroids.

Determination of terpenoids

The extract (5 mL) of each extract was added to a carefully prepared mixture of 1 mL chloroform and 1 mL concentrated sulphuric acid. The appearance of a reddish brown colour in the boundary emphasized the existence of terpenoids.

Test of anthraquinones

Plant powdered sample (1 g) was heated to boil with 10 mL of 0.5 M KOH including 1 mL of 3% H₂O₂ solution. The blend was boiled, cooled, filtered, and to 5 mL of filtrate few quantity of glacial acetic acid was added. The acidified blend was extracted with 10 mL of benzene and allowed to stand. A 5 mL of the benzene solution was mobilized with 3 mL of 10% ammoniumhydroxide solution and the benzene layer was left to stand. The presence of anthraquinones was indicated by pink or red colour.

Test of flavonoids

To 3 mL of the filtrate, 0.5 mL of concentrated hydrochloric acid was added, followed by adding of few magnesium ribbons. Appearance of pink tint represents the occurrence of flavonoids.

Test of saponin

The ground specimens (2.0 g) was mixed with 20 mL of distilled water and heated in water bath and filtered. Ten ml of the filtrate was blended with 5.0 mL of distilled water and stirred powerfully for stable permanent foam. Few drops of olive oil were added to the foam and the blend was strongly shaken to obtain an emulsion.

Antimicrobial Assay

Bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and Fungi (*Candida albicans*) were used in this investigation. Antimicrobial activity of the previous considered extracts were run using the agar diffusion method depicted by Collins and Lyne, (1970). All the above remained bacteria were inoculated into nutrient agar medium and fungi inoculated to potato dextrose agar medium. The well of 8 mm diameter was dug in the culture medium using sterile cork borer. The collected extracts were intensified and filled into sterile readymade discs (Hi-media, Bombay) in various volumes of 6.25, 12.25, 25, 50, and 100 µl/disc respectively and left to dry for one day at 25°C. The agar plates were expanded with 100 µl of actively growing broth cultures of the special bacteria and are left to drain off for 10 minute. The sterile available discs filled with each extract distributively (6.25, 12.25, 25, 50, and 100 µl/disc respectively) were enjoin on the vaccinated plates. Different extracts were run to capacity in each well. Culture plates were brooded at 37°C for one day in bacteria and incubated at 37°C for 4 days in fungi. Bioactivity was specified by scale diameter of inhibition zones in mm. Solvents used for extraction render as control⁹.

Statistical analysis

Statistical comparisons were carried out by one-sample t-test analysis using SPSS Statistics version 16. Differences were significant at the level of $p \leq 0.05$. All the data are reported as means \pm standard deviation (SD) of triplicate determinations ($n = 3$).

RESULT AND DISCUSSION

The chemical analysis

Tests for active ingredients were performed on all the studied leaf extracts of *Dodonaea viscosa* and *Capparis spinosa*, the results were reported in Table 1. Which explained that the leaves extracts of *D. viscosa* of all fractions (petroleum ether,

chloroform, ethyl acetate, n-butanol and ethanol) exhibited the occurrence of phenolics, flavonoids, tannins, terpinoid, and carbohydrate while the ethyl acetate and n-butanol fraction showed the absence of the alkaloids. Saponin was missed in petroleum ether fraction. Furthermore in Table 2 (leaves extracts of *C. spinosa*), flavonoids, tannin, and carbohydrate were occurred in n-butanol, ethyl acetate, chloroform and ethanol fractions. Flavonoid, tannin, saponin, and carbohydrate were not detected in petroleum ether extract of *C. spinosa*. These results differ from those obtained by (Riaz *et al.*; 2012) whose revealed the presence of tannin only in ethyl acetate and n-butanol in *Dodonaea viscosa* leaf extracts¹, whereas in this study tannin was present in all fractions but the two studies agreed upon the presence of terpinoid in all fractions. (El-Sawi *et al.*; 2018) confirmed that alkaloid was absent in the leaf extract of *D. viscosa* whereas flavonoid, tannin, saponin, steroid and carbohydrate were present in moderate concentration¹⁵. whilst in this study all classes of natural products were present in moderate to high concentrations. On the other hand (Ramamurthy *et al.*; 2018) their results came in accord with result of this study⁹.

(Tagnaout *et al.*, 2016). Studied the chemical constituents of *Capparis spinosa* leaf extracts in different solvents⁶. The results obtained by them were considerably different from what we found. They revealed the absence of tannin and saponin in the plant extract however we found them in our study particularly in ethyl acetate, n-butanol and ethanol extracts. At the same time (AL-Azawi *et al.*, 2018), obtained the same results as we found in our study in all extracts of *C. spinosa*¹⁶. This reflect the effect of the climate in the chemical constituents and in their concentrations in the plants that grow wildly in different environments. Also represent the influence of the method of the extraction in the same aspect. The results in Table 1 and 2 exhibited that *Dodonaea viscosa* extracts were rich with Flavonoids and phenolic compounds than that detected in the homologous extractors of *C. spinosa*, which mean that *D. viscosa* was very rich of antioxidants compounds and is an excellent source for these compounds. These results supported and confirmed the utilize of these medicinal plants as traditional medicine for remedy of many ailments.

Table 1: Results of chemical analysis of *Dodonaea viscosa* leaf extracts

Extract	Flavonoid	Tannin	Alkaloid	Terpinoid	Saponnin	Carbohydrate
Petroleum ether	+	+	+	+	-	+
Chloroform	++	++	++	++	-	+
Ethylacetate	+++	+++	-	++	+++	++
n-butanol	+++	++	-	+	++	+++
Ethanol	++	+++	++	+	++	++

+ = positive, ++ = good present, +++ = strongly present, - = not detected

Table 2: Results of chemical analysis of *Capparis spinosa* leaf extracts

Extract	Flavonoid	Tannin	Alkaloid	Saponnin	Carbohydrate
Petroleum ether	-	-	+	-	-
Chloroform	+	+	++	-	+
Ethylacetate	+++	++	+	++	+
n-butanol	+++	++	-	+	+++
Ethanol	+	+	-	+	++

+ = positive, ++ = good present, +++ = strongly present, - = not detected

Antimicrobial Activity

Maximum zone of inhibition for 100 mg/mL was in recorded by the ethyl acetate extract of *Dodonaea viscosa* leaf was 30 mm against *Bacillus subtilis*. Similarly 20 mm were registered by ethyl acetate of *Capparis spinosa* leaf extract was observed against *Bacillus subtilis*. Chloroform extract of the two species showed no efficacy against all test bacteria organisms except *Bacillus subtilis* (15 mm). Ethyl acetate and n-butanol extracts exhibited high to moderate activity 10-30 mm

against all test bacteria organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus agalactiae*) by the leaves extracts of both species (Table 3 and 4). Salama *et al.*, 2018 reported that *Dodonaea viscosa* leaf extracts showed moderate to low activity against various tested pathogens (zone of inhibition in the range 7 – 10 mm) whereas we found strong antibacterial activity in our study (30–9 mm). In this search, *Staphylococcus aureus* showed higher response for the extracts of *D. viscosa* except chloroform extract

and this report is similar to that of (Udaya prakash *et al.*, 2012)¹⁷. But the record result for *Bacillus subtilis* is differ, as Udaya prakash *et al.* found that *B. subtilis* was affected with all extracts we found n- butanol extract not influenced. More over AL-Azawi *et al.*,¹⁶ obtained different results, they reported that, *Staphylococcus aureus* and *Escherichia coli* reflected high response to *C. spinosa* leave extracts whereas *Pseudomonas aeruginosa* was not affected but we found *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed high to moderate response respectively whilst *Escherichia coli* exhibit moderate response. This may be due to the occurrence of terpenes, phenols, flavonoids, saponins, in n-butanol and ethyl acetate extracts, which have a great effect as antimicrobial agents (Table 1 and 2)¹⁰. Ethyl acetate and n-butanol leaves extractors of *D. viscosa* and *C. spinosa* showed moderate effect against fungi *C. albicans*, the zone of inhibitions for 100 mg/mL were (18, 10 and 17, 10 mm respectively).

Escherichia coli showed good susceptibility towards the ethyl acetate and n-butanol leave extracts of *Dodonaea viscosa*, where the MIC were 25, and 12.25 mg/mL respectively and for the *Capparis spinosa*, the MIC were 25, 25 mg/mL (Table 3, 4). These extracts for the two plant species have a pronounced effect on the outgrowth of *S. aureus*, *B. subtilis* and *C. albicans*, the MIC are 6.25, 12.5, 25, 25, 12.5, and 50 for *Dodonaea viscosa* and are 25, 25, 50, 50, 25, and 25, 50 mg/mL for *Capparis spinosa* (Table 5, 6). These results supported the work that has been summarized by Rojas *et al.*, 1992; Mothana *et al.*, who announced that the ethyl acetate extracts of *D. viscosa* and *Capparis spinosa* have antimicrobial effects^{11,12}. The response of the five microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans*) towards *Dodonaea viscosa* leave extracts were represented in Figure 1.

Table 3: Results of zone of inhibition (mm) of *Dodonaea viscosa* leave extracts

Extract	Extract volume μ l /disc	Microorganism				
		Ec	Pa	Sa	Bs	Ca
		Diameter of inhibition zone (mm)				
Chloroform	100	-	-	-	14.9 \pm 0.14	-
	50	-	-	-	14.3 \pm 0.49	-
	25	-	-	-	10.1 \pm 0.21	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
Ethyl acetate	100	24.8 \pm 0.25	-	20.4 \pm 0.63	30.4 \pm 0.63	18.4 \pm 63
	50	18.2 \pm 0.28	-	20.1 \pm 0.21	20.3 \pm 0.49	17.7 \pm 0.35
	25	9.8 \pm 0.28	-	15.3 \pm 0.42	20.3 \pm 0.42	15.1 \pm 0.14
	12.5	-	-	15.4 \pm 0.40	-	14.7 \pm 0.42
	6.25	-	-	9.9 \pm 0.35	-	-
n- Butanol	100	14.9 \pm 0.14	13.9 \pm 0.07	9.8 \pm 0.28	-	10.2 \pm 0.35
	50	13.1 \pm 0.14	10.2 \pm 0.35	10.1 \pm 0.14	-	9.8 \pm 0.28
	25	9.5 \pm 0.70	10.2 \pm 0.28	10.4 \pm 56	-	-
	12.5	9.9 \pm 1.27	9.5 \pm 0.70	10.3 \pm 0.49	-	-
	6.25	-	-	-	-	-

Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Bs: *Bacillus subtilis*, Ca: *Candida albicans*, and - : no inhibition zone. The results were reported as mean \pm SD (n =3).

Table 4: Results of zone of inhibition (mm) of *Capparis spinosa* leave extracts

Extract	Extract volume μ l /disc	Microorganism				
		Ec	Pa	Sa	Bs	Ca
		Diameter of inhibition zone (mm)				
Chloroform	100	-	-	-	13.3 \pm 0.42	-
	50	-	-	-	10.3 \pm 0.49	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
Ethyl acetate	100	18.7 \pm 0.49	18.1 \pm 0.07	16.1 \pm 0.14	19.8 \pm 0.28	16.8 \pm 0.21
	50	16.9 \pm 0.07	15.9 \pm 0.14	14.6 \pm 0.84	18.2 \pm 0.21	13.5 \pm 0.70
	25	14.9 \pm 0.14	10.1 \pm 0.14	9.8 \pm 0.28	15.3 \pm 0.35	10.2 \pm 0.28
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
n- Butanol	100	16.3 \pm 0.35	14.9 \pm 0.07	10.1 \pm 0.21	-	9.9 \pm 0.14
	50	12.9 \pm 0.07	12.1 \pm 0.14	9.1 \pm 0.07	-	10 \pm 0.00
	25	9.9 \pm 0.21	10.1 \pm 0.07	-	-	-
	12.5	-	9.9 \pm 0.14	-	-	-
	6.25	-	-	-	-	-

Table 5: Results of Minimum Inhibitory Concentration (MIC) (mg/mL) of *Dodonaea viscosa* leaf extracts

Extract	Concentration of extract in mg/ml	Microorganism				
		Ec	Pa	Sa	Bs	Ca
		(MIC) (mg/ml)				
Chloroform	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	25	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
Ethyl acetate	100	-	-	-	-	-
	50	-	-	-	-	-
	25	25	-	6.25	25	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
n- Butanol	100	-	-	-	-	-
	50	-	-	-	-	-
	25	12.50	12.50	12.50	-	50
	12.50	-	-	-	-	-
	6.25	-	-	-	-	-

Table 6: Results of Minimum Inhibitory Concentration (MIC) (mg/mL) of *Capparis spinosa* leaf extracts

Extract	Concentration of extract in mg/ml	Microorganism				
		Ec	Pa	Sa	Bs	Ca
		(MIC) (mg/ml)				
Chloroform	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	25	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
Ethyl acetate	100	-	-	-	-	-
	50	-	-	-	-	-
	25	25	25	25	25	25
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
n- Butanol	100	-	-	-	-	-
	50	-	-	-	-	-
	25	12.5	-	50	-	50
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-

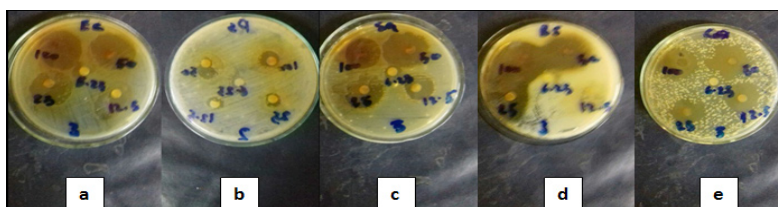


Fig. 1. The effect of the chloroform, ethyl acetate, and n-butanol leaves extracts of *D. viscosa* on test organisms (Bacteria and Fungi): a: *Escherichia coli*, b: *Pseudomonas aeruginosa*, c: *Staphylococcus aureus*, d: *Bacillus subtilis*, e: *Candida albicans*

CONCLUSION

The findings of this study suggest the importance of *D. viscosa* and *C. spinosa* L species for use in pharmacy and phytotherapy. Actually, phytochemical screening reveals the richness of the

plants by secondary metabolites that have alimentary and medicinal important for human being healthy because most of the plants extracts constitute the active ingredients such as alkaloids, flavnoids, terpenoids, steroids, saponins, and tannin. Moreover, *D. viscosa* and *C. spinosa* L can constitute an

interesting source of natural antioxidants towards the free radicals in comparison with other provenances. In the present research, MIC of the extracts showed relatively best activities in all the tests obviously caused by effective extraction of phytochemicals. It is concluded on this study that n-butanol, and ethyl acetate extracts (leaves of *D. viscosa* and *C. spinosa*) from Jabal Shuda/South Saudi Arabia have obvious effect on the tested microorganisms, and these results as compared with other studies, indicated that the type of solvents and procedure of extraction of *D. viscosa* and *Capparis spinosa* significantly affects the quantity of the chemical components, thus some antimicrobial effects differ from that reported in the literature.

Antimicrobial activity versus *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus*

aureus, *Bacillus subtilis*, and *Candida albicans* exhibited that the plant can be used in the curing of gastrointestinal infection and diarrhea in human. This may be referred to its containment in phytochemical components like alkaloids, flavonoids, saponins, tannins, amino acids and carbohydrates, which were also reported by Ikewuchi *et al.*.

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Conflicts of interest

The authors announce that there are no conflicts of interest related to this article.

REFERENCES

- Riaz, T.; Abbasi, M. A.; Aziz-ur-Rehman, S. T.; Ajaib, M.; Khan, K. M. *J. Serb. Chem. Soc.*, **2012**, *77*(4), 423–435. Doi:10.2298/JSC11061183R.
- Gull, T.; Sultana, B.; Bhatti, I. A.; Jamil, A. *Int. J. Agric. Biol.*, **2015**, 1814–9596. Doi:1017957/IJAB/14.0007.
- Ramzi, A.; Mothana, A.; Salah, A.; Abdo, A.; Sidgi, H.; Faisal, M.; Althawab, N.; Sama, A.; Alaghbari, Z.; Ulrike, L. *Adv. Acc. Pub.*, **2010**, *7*(3), 323–330. Doi:10.1093/ecam/nen004.
- Shafek, R. E.; Shafik, N. H.; Michael, H. N.; El-Hagrassi, A. M.; Osman, A. F. *J. Chem. Pharm. Res.*, **2015**, *7*(5), 109-116.
- Al-Snafi, A. E. *IOSR J. Pharm.*, **2017**, *7*(2), 10-21. Doi:10.9790/3013-0702011021.
- Tagnaout, I.; Zerkani, H.; Mahjoubi, M.; Bourakhouadar, M.; Alistiqsa, F.; Bouzoubaa, A.; Zair, T. *J. Pharmacogn. Phytochem.*, **2016**, *8*(12), 1993-200.
- Harborne, J. B., 3rd Edition, Chapman and Hall Ltd., London., **1973**, 49-188.
- Zohra, S. F.; Meriem, B.; Samira, S.; Muneer, M.S.A. *J. Nat. Prod. Plant Resour.*, **2012**, *2*(4), 512-516.
- Rama Murthy, V.; Maria Rajeswari, D; Gowri, M. K.; Jayanthi, G.; raveendran, S. *J. Pure Appl. Zool.*, **2013**, *1*(2), 178-184,
- Ekwenye, U. N.; Elegalam, N. N. *J. Mol. Adv Sci.*, **2005**, *1*(4), 411-416.
- Rojas, A.; Hernandez, L.; Pereda, M. R.; Mata, R. *J. Ethnopharmacology.*, **1992**, *35*(3), 275-283. Doi:10.1093/ecam/nen004.
- Mothana, R. A. A.; Abdo, S. A. A.; Hasson, S.; Althawab, F. M. N.; Alaghbari, S. A. Z.; Lindequist, U., **2008**.
- Moideen, M. M. J.; Raffick, M. M. *Int. J. Phytopharmacology.*, **2012**, *3*(1), 21- 26.
- Ikewuchi, C. C.; Ikewuchi, C.J.; Ayalegy, O.E.; Onyeike, N.E. *J. Appl. Sci. Environ Manage.*, **2010**, *14*, 103-106.
- El-Sawi, S. A.; Motawe, H. M.; Ahmad, S. S.; Ibrahim, M. E. *J. Mater. Environ. Sci.*, **2018**, *9*(5), 1495-1502. Doi.org/10.26872/jmes.2018.9.5.164.
- AL-Azawi, A. H., Ghaima, K. K., Salih, H. H. *Bioscience Research.*, **2018**, *15*(3), 2611-2618. online freely at www.isisn.org.
- Udaya prakash, N.K.; Selvi, C.R.; Sasikala, V.; Dhanalakshmi, S.; Bhuvaneshwari udaya, P. S. *International Journal of Pharmacy and Pharmaceutical Sciences.*, **2012**, *4*(2), 509-512.