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Insecticidal, antimicrobial and antioxidant activity and elemental analysis of *Cochlospermum religiosum* (L.) Alston (Bixaceae)

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ABSTRACT

Objectives: *Cochlospermum religiosum* (L.) Alston is one of the extensively used medicinal plant belonging to the family Bixaceae. In the present study, we determined antimicrobial, antioxidant, and insecticidal activity and elemental analysis of *C. religiosum* flowers.
Methods: Shade dried and powdered flower material was extracted by maceration process using methanol. Antibacterial activity of flower extract was determined by agar well diffusion assay against gram positive and gram negative bacteria. Antifungal activity was tested against two molds namely *Rhizopus* sp. and *Curvularia* sp. by poisoned food technique. Antioxidant activity was evaluated by DPPH free radical scavenging and ABTS free radical scavenging assays and ferric reducing assay. Insecticidal activity was assessed in terms of larvicidal activity against I, II and III instar larvae of *Aedes* species and *Anopheles* species. Elemental analysis was carried out to estimate the content of major and minor elements.
Results: The flower extract was effective in inhibiting all test bacteria. Overall, the flower extract was effective against gram positive bacteria to higher extent when compared to gram negative bacteria. Flower extract showed dose dependent scavenging of DPPH and ABTS radicals with an EC_{50} value of 2.72 and 1.50µg/ml, respectively. In ferric reducing assay, an increase in the absorbance with increase in concentration indicated reducing potential of flower extract. At 1mg/ml concentration, the flower extract caused 100% mortality of I, II and III instar larvae of *Aedes* species and *Anopheles* species. The flower was shown to contain potassium and iron in highest quantity among major and minor elements, respectively while magnesium and chromium content was least among major and minor elements, respectively.
Conclusions: The results are promising and the study highlights the possible utilization of the *C. religiosum* flowers against pathogenic microorganisms and oxidative stress and to manage mosquito-borne diseases. The flower can be used as a food supplement as it is shown to contain various mineral elements that are required.

Key words: *Cochlospermum religiosum*, Maceration, Agar well diffusion assay, DPPH, ABTS, Ferric reducing

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INTRODUCTION

Insect pests are troublesome to plant as well as animal health. Among various arthropod vectors, mosquitoes are of high significance. Mosquitoes are responsible for transmission of dreadful diseases such as malaria, dengue, chikungunya, filariasis, yellow fever and Japanese encephalitis. The management of mosquito borne disease usually employs the application of synthetic insecticides. Although proven effective, however, their indiscriminate application for vector control resulted in emergence of resistant strains of mosquitoes as well as deleterious effect on environment. Moreover, the cost of insecticides is high and there is an increased risk of health hazard due to their ill effect on the health of humans. Screening botanicals for insecticidal activity has been intensified to overcome the adverse effects of synthetic chemicals. It is shown that crude extracts as well as purified compounds from several plant species exhibit insecticidal activity against various insect pests including mosquitoes which spread dreadful human diseases such as dengue and malaria[1-15].

Antibiotics are promising in terms of their potential application in the therapy against infectious diseases. The discovery of antibiotics has revolutionized the field of chemotherapy. However, indiscriminate use of these agents resulted in the emergence of resistant strains of bacteria in both community and hospital settings. Bacteria such as *Escherichia coli*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are considered to be few among the antibiotic resistant bacteria. Moreover, the ability of these pathogens to transmit the resistance trait to susceptible strains through horizontal transfer seems to be even more serious issue.

Hence, there is an upsurge interest in finding alternatives with low or no drawbacks. Natural products, including plants and their metabolites, are considered to be one of the potential alternatives for disease therapy. Studies have shown that extracts and isolated compounds from higher plants exhibit marked antibacterial potential against a range of pathogenic bacteria including drug resistant strains[16-21].

Fungi are well known as the causal agents of plants and animals since time immemorial. They cause huge number of diseases in plants and many diseases in animals and humans. Management of fungal infections of plants is usually carried out by the use of synthetic fungicides. Infections caused by clinical pathogens are usually treated by the use of antifungal drugs such as azoles. It has been well established that the fungal pathogens have gained resistance to most commonly used fungicides as wells as antifungal agents for clinical pathogens. Besides, high cost, deleterious effect on environment, and development of resistance against fungicides as well as antifungal agents also limits the application of these agents. Natural products, including plants and their metabolites, have been promising with respect to their potential to inhibit a range of phytopathogenic fungi as well as clinical isolates. The use of plant based formulations is cheaper, safer and is not usually subjected to resistance development in pathogenic fungi[17,22-27].

A free radical is an atom or a molecule that has an unpaired electron in an outer shell. Free radicals are highly reactive and are known to damage lipids, nucleic acids and proteins leading to cellular damage. The excessive production of free radicals leads to oxidative stress condition which is implicated in several pathophysiological conditions including aging, cancer, cardiovascular diseases and neurodegenerative diseases. In oxidative stress condition, the endogenous antioxidant system of the body will not be able to completely inactive the free radicals generated by their excessive production. Hence, there is an extra demand for antioxidants in the form of diet. Plants and their metabolites are shown to be promising resources of natural antioxidant principles. Phytochemicals, in particular phenolic compounds and flavonoids, are shown to scavenge free radicals more efficiently and thereby alleviate oxidative damage induced by excessive generation of free radicals[20,28-33].

Cochlospermum religiosum (L.) Alston (synonym *C. gossypium* DC) is one of the medicinal tree species belonging to the botanical family Bixaceae (Figure 1). The plant is known by the names Golden silk cotton tree/butter cup tree in English, Gabdi in Hindi, Girisalmalika in Sanskrit, and Arasina buruga in Kannada. In India, *C. religiosum* is grown near temples because of the bright yellow flowers that are to be used for offerings to god and also for aesthetic purpose. *C. religiosum* is traditionally used in syphilis, gonorrhea, trachoma, cough, jaundice etc. The gum katira or gum kondagogu, obtained from the stem, is used for treating diarrhea, dysentery, pharyngitis, eye problems, asthma and stomachache. Besides, the plant finds a wide range of ethnoveterinary use[34-43]. When literature survey was carried out, it was found that no much work is done on the biological activities of flower of *C. religiosum*. In the present study, we estimated mineral elements and screened insecticidal antibacterial and antioxidant activity of flowers of *C. religiosum*.

Figure 1: ***Cochlospermum religiosum***

MATERIALS AND METHODS

Collection and extraction of plant material

The plant material (flower) was collected near Hosagunda, Shivamogga district, Karnataka, India during 2019 and authenticated on the basis of its characteristics by using standard flora[44]. Extraction of shade dried and powdered flower was carried out by maceration technique using methanol in a stoppered container[45]. The yield of extract obtained was 14.08%.

Insecticidal activity of flower extract

The insecticidal potential of flower extract (1mg extract/ml of water) was evaluated in terms of its larvicidal activity against I instar, II instar and III instar larvae of *Aedes* species (Diptera: Culicidae) and *Anopheles* species (Diptera: Culicidae). Mortality of larvae and pupae were recorded after 24 hours[4].

Antibacterial activity of flower extract

Agar well diffusion method, as described in the study of Ankith et al.[45], was used to evaluate antibacterial activity of flower extract (25mg extract/ml of dimethyl sulfoxide [DMSO]) against two gram positive and six gram negative bacteria. Streptomycin was used as reference antibiotic (1mg/ml of sterile distilled water). DMSO was used as negative control. Zones of inhibition formed around the wells were taken positive for antibacterial activity.

Antifungal activity of flower extract

Poisoned food technique, as described in the study of Raghavendra et al.[46], was used to determine antifungal activity of flower extract (1mg extract/ml of potato dextrose agar medium) against two fungi viz. *Rhizopus* sp. and *Curvularia* sp. A reduction in the colony diameter of test fungi in poisoned plates when compared to control plates was considered as antifungal activity. The extent of inhibition of fungal growth in poisoned plates was calculated using the formula:

Reduction in mycelial growth (%) = [Dc – Dt / Dc] x 100, where Dc and Dt represents diameter of fungal colonies in control and poisoned plates, respectively.

Antioxidant activity of flower extract

2,2-diphenyl-1-picrylhydrazyl [DPPH] radical scavenging assay

Scavenging potential of different concentrations of flower extract namely 0.78-50µg/ml was evaluated by DPPH radical scavenging assay as described by Raghavendra et al.[46]. Butylated hydroxyanisole (BHA) and ascorbic acid were used as reference standards. The absorbance was measured at 520nm. The extent of scavenging of radicals was calculated using the formula:

Scavenging of DPPH radicals (%) = [Ac – At / Ac] x 100, where Ac and At represents absorbance of DPPH control and absorbance of DPPH in the presence of extract/standard, respectively. EC_{50} values were calculated.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) [ABTS] scavenging assay

Various concentrations of flower extract, namely 0.78-50µg/ml, were screened for antiradical activity by ABTS radical scavenging assay. Ascorbic acid and BHA were used reference standard. The absorbance of each tube was measured at 734nm[46]. The extent of scavenging of radicals was calculated using the formula:

Scavenging of ABTS radicals (%) = [Ac – At / Ac] x 100, where Ac and At represents absorbance of ABTS control and absorbance of ABTS in the presence of extract/standard, respectively. EC_{50} values were calculated.

Ferric reducing assay

The reducing potential of flower extract (0.78-50µg/ml of methanol) was evaluated by ferric reducing assay as described in the study of Raghavendra et al.[46] The absorbance of reaction mixture in each of the tube was read at 700nm. Ascorbic acid was used as reference standard. An increase in the absorbance with increase in concentration indicates reducing power.

Mineral analysis of *C. religiosum* flower

Prior to estimation of mineral elements, the flower powder was subjected to digestion using mixture of nitric acid and perchloric acid. The digested sample was used to estimate the content of major elements and minor elements by various protocols. Sodium (Na) and potassium (K) were estimated by using flame photometer (Systronics, Flame Photometer 128). Phosphorus (P) was estimated by spectrophotometric method. Nitrogen (N) estimation was carried out by Kjeldahl procedure. Elements namely copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), calcium (Ca), chromium (Cr), nickel (Ni), magnesium (Mg) were estimated using atomic absorption spectrometer (PerkinElmer PinAAcle 900F).

RESULTS AND DISCUSSION

Insecticidal activity of *C. religiosum* flower extract

Management of mosquito borne diseases can be accomplished by targeting various stages of life cycle of mosquitoes namely eggs, larvae, pupae and adult form. Among these, targeting the larval stage (especially in stagnant water which seems to be a breeding ground for mosquitoes) and prevention of their further development into pupae and adult seems to be one of the widely use approach for disease prevention. Studies have shown that botanicals exhibit insecticidal activity in terms of ovicidal, oviposition deterrent, larvicidal, pupicidal, and repellant activities. Plant extracts and plant secondary metabolites have been considered as potential alternatives for chemical insecticides[4,5,47-55]. In the present study, insecticidal activity of flower extract was evaluated against I, II and III instar larvae of *Anopheles* sp. and *Aedes* sp. and the mortality of larvae and pupae was recorded at the end of 24 hours of exposure. The flower extract was highly effective in causing 100% mortality of I, II and III instar larvae of both mosquito species at 1mg/ml concentration (Table 1).

Table 1: Larvicidal activity of flower extract of *C. religiosum*

Mosquito	Larval stage	Number of larvae/pupae	Number of dead larvae/pupae	Mortality (%)
Anopheles species	I instar	10	10	100.00
	II instar	10	10	100.00
	III instar	10	10	100.00
Aedes species	I instar	10	10	100.00
	II instar	10	10	100.00
	III instar	10	10	100.00

Antibacterial activity of flower extract

Intensified study by scientific community on botanicals with antibacterial activity is triggered due to failure of antibiotics to act against pathogenic bacteria because development of resistance in pathogens. Plant extracts and plant secondary metabolites are shown to be promising alternatives for disease therapy[16,56,57,58]. In the present study, the flower extract of *C. religiosum* was effective in inhibiting the growth of all bacteria as evidence by the presence of zones of inhibition around the wells (Table 2). Among bacteria, marked inhibitory activity of flower extract was observed against gram positive bacteria. The gram positive bacteria viz. *S. aureus* and *B. subtilis* were inhibited to almost similar extent. Among gram negative bacteria, *E. coli* and *X. campestris* were inhibited to highest and least extent respectively. The susceptibility of gram negative bacteria to extract was in the order: *E. coli* > *P. aeruginosa* > *P. syringe* > *S. typhi* > *K. pneumoniae* and *X. campestris*. Reference antibiotic strongly inhibited test bacteria when compared to flower extract. DMSO did not cause inhibition of bacteria. Earlier studies by Panda et al.[59], Zingare[60], Goud et al.[61], Bai et al.[62], Ponnamma et al.[63] and Kawde et al.[64] revealed antibacterial activity of different parts of *C. religiosum*. However, Pumpaluk et al.[65] showed that extract of seeds/fruits was not effective against cariogenic bacteria *Streptococcus mutans*, *Lactobacillus casei* and *Actinomyces viscosus*.

Table 2: Antibacterial activity of flower extract of *C. religiosum*

Test bacteria	Zone of inhibition in cm		
	Flower extract	Streptomycin	DMSO
S. aureus	2.60±0.00	3.50±0.00	0.00±0.00
B. subtilis	2.56±0.05	3.30±0.00	0.00±0.00
E. coli	2.53±0.05	3.53±0.05	0.00±0.00
P. aeruginosa	2.30±0.00	2.60±0.00	0.00±0.00
P. syringe	2.03±0.05	2.80±0.10	0.00±0.00
K. pneumoniae	1.80±0.10	2.13±0.05	0.00±0.00
X. campestris	1.43±0.05	2.53±0.05	0.00±0.00
S. typhi	2.00±0.00	3.30±0.00	0.00±0.00

Antifungal activity of flower extract

Extensive use of synthetic fungicides caused havoc in the environment due to their residual effect and deleterious effect on non-target organisms. Besides, development of resistance to fungicides is another serious issue. Many of human pathogenic fungi have developed resistance against most commonly used antifungal drugs. Plant extracts, plant based formulations and purified metabolites from plants are shown to be promising alternatives for human and plant pathogenic fungi[66-71]. The result of antifungal activity of flower extract is shown in Table 3. The extract was effective in causing suppression of mycelial growth of test fungi to considerable extent. An inhibition of >40% of growth was observed in case of both fungi. Among fungi, *Rhizopus* sp. was inhibited to slightly higher extent (49.57% inhibition) when compared to *Curvularia* sp (42.49% inhibition). In an earlier study, the methanolic and aqueous extracts of leaves of *C. religiosum* were shown to display dose dependent inhibitory activity against *Alternaria alternata*, *Chaetomium globosum* and *Fusarium oxysporum*[72]. In another study by Goud et al.[61], the stem bark extract of *C. religiosum* failed to produce antifungal activity against *Aspergillus niger*.

Table 3: Antifungal activity of flower extract of *C. religiosum*

Treatment	Colony diameter in cm (% inhibition)	
	***Curvularia* sp.**	***Rhizopus* sp.**
Control	3.53±0.05	7.00±0.00
Flower extract	2.03±0.05	3.53±0.05

DPPH free radical scavenging activity of flower extract

The method of scavenging of DPPH radicals is one of the most widely used in vitro antiradical assays. The method is simple, accurate and the results obtained are reproducible. The substances (antioxidants) reduce the purple colored DPPH radicals into yellow colored DPPHH (diphenylpicryl hydrazine). The method is extensively used to evaluate antiradical activity of plant extracts[20,30,33,73-76]. In the present study, the flower extract of *C. religiosum* was shown to exhibit marked dose dependent scavenging of DPPH radicals (Figure 2) as evidenced by bleaching of purple color of radical to yellow color. A scavenging activity of 50% and higher was shown by concentration viz. 3.12, 12.50 and 1.56µg/ml of flower extract, ascorbic acid and BHA, respectively. The flower extract exhibited marked activity (EC_{50} value 2.72µg/ml) when compared to ascorbic acid (EC_{50} value 4.52µg/ml), however, the activity of flower extract was slightly lesser than that of BHA (EC_{50} value 2.43µg/ml). In earlier studies, the gum[77], stem bark[64] and leaves[63] were shown to exhibit antiradical activity in DPPH assay.

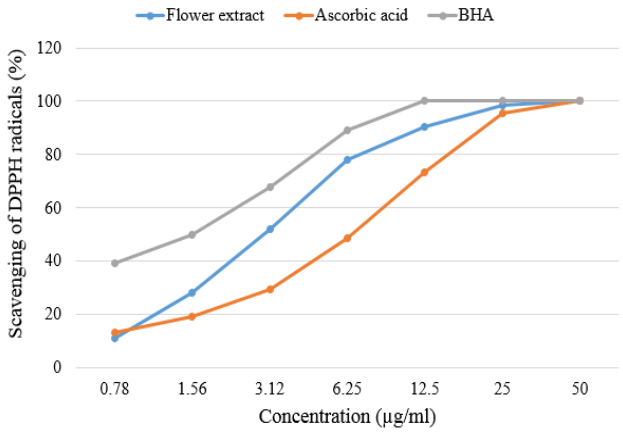

Figure 2: Scavenging of DPPH radicals by *C. religiosum* flower extract

ABTS free radical scavenging activity of flower extract

ABTS radical scavenging assays is yet another widely used in vitro antiradical assay being used commonly for evaluating radical scavenging nature of plant extracts. It differs from DPPH assay in that the ABTS radical has to be generated prior to assay and is done by mixing ABTS salt solution with an oxidizing agent such as potassium persulfate. In this assay, substances (compounds) having electron donating potential will reduce the blue-green colored ABTS radical solution to colorless neutral form[30,73,74,75,76,78,79]. In the present study, antiradical activity of flower extract of *C. religiosum* was also tested by ABTS radical scavenging assay. The flower extract was shown to exhibit concentration dependent scavenging of ABTS radicals (Figure 3). A scavenging activity of 50% and higher was observed at concentration 3.12, 3.12 and 1.56µg/ml of flower extract, ascorbic acid and BHA, respectively. Flower extract scavenged ABTS radicals more efficiently with an EC_{50} value of 1.50µg/ml when compared to ascorbic acid (EC_{50} value of 3.32µg/ml), however, the activity of flower extract was slightly lesser than that of BHA (EC_{50} value of 1.42µg/ml).

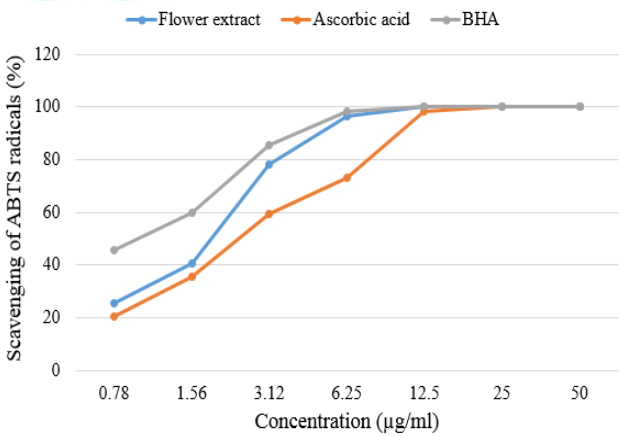

Figure 3: Scavenging of ABTS radicals by *C. religiosum* flower extract

Reducing activity of flower extract

The reducing capacity of an extract can be attributed to the presence of reductones and the presence of reductants (antioxidants) in the extract would result in the reduction of Fe^{+3} to Fe^{+2} by donating an electron. This reducing ability of an extract or the compound may serve as a significant indicator of its potential antioxidant activity. Ferric reducing assay is widely used to determine antioxidant activity of

plant extracts[20,80-83]. The reducing potential of flower extract was evaluated by ferric reducing assay. An increase in the absorbance was observed with an increase in the concentration of flower extract and ascorbic acid which indicates the reducing efficacy (Figure 4). In an earlier study by Bai et al.[62], the methanolic extract of leaves of *C. religiosum* failed to exhibit antioxidant activity by ferric reducing assay.

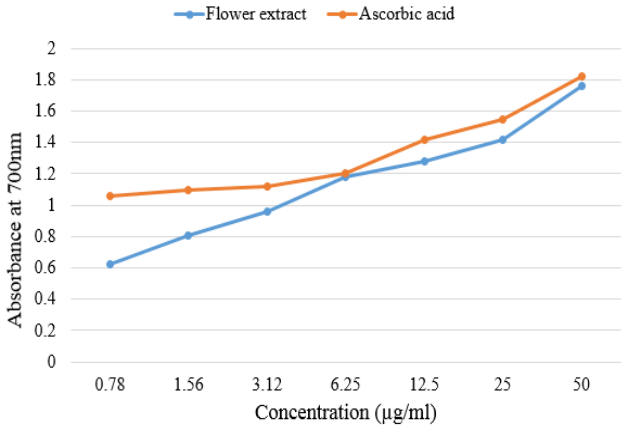

Figure 4: Reducing activity of *C. religiosum* flower extract

Mineral content of *C. religiosum* flower

Every individual need macronutrients and micronutrients in balanced proportion to lead healthy life. Nutrients such as carbohydrates, proteins and lipids form the major portion of the diet while nutrients such as mineral elements and vitamins are required in smaller proportion. Elements namely N, P, K, Ca, Mg and Na are considered to be the major elements as they are required in greater quantity while Fe, Mn, Zn, and Cu, are required in small quantity and hence, referred as minor elements. Although required in minor concentration, these mineral nutrients play indispensable role in the physiology of an individual. The absence of insufficiency of these elements results in some deficiency symptoms[84-88]. In the present study, we determined the quantity of major and minor elements in the flower of *C. religiosum* by various analytical methods and the result is shown in Table 4. Among major elements, the content of potassium was highest (1.533%) while the content of sodium was least (0.036%). Among minor elements, the content of iron (333.60ppm) and chromium (1.65ppm) was highest and least, respectively.

Table 4: Content of major and minor minerals in the flower

Major element	Quantity	Minor element	Quantity
Nitrogen (%)	1.020	Iron (ppm)	333.60
Phosphorous (%)	0.174	Manganese (ppm)	38.50
Potassium (%)	1.533	Zinc (ppm)	21.20
Calcium (%)	0.260	Copper (ppm)	10.80
Magnesium (%)	0.070	Chromium (ppm)	1.65
Sodium (%)	0.036	Nickel (ppm)	3.95

CONCLUSIONS

The study revealed potent antibacterial, antifungal, antiradical, ferric reducing and insecticidal activity of *C. religiosum* flower extract. The flower extract, in suitable form, can be used against microbial infections and oxidative damage induced by free radicals. In suitable formulation, the plant can be exploited for the management of mosquito-borne diseases by interrupting the life cycle of mosquito vectors. The flower may be incorporated as food supplement as it is shown to contain marked quantity of major and minor mineral elements. Further in depth studies are merited in order to recover active principles from flower extract and to investigate their biological activities.

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None

CONFLICTS OF INTEREST

None declared

REFERENCES

1. Mittal PK, Subbarao SK. Prospects of using herbal products in the control of mosquito vector. ICMR Bulletin 2003; 33(1):1-10.
2. Chowdhury N, Laskar S, Chandra G. Mosquito larvicidal and antimicrobial activity of protein of *Solanum villosum* leaves. BMC Complementary Altern Med 2008; 8:62.
3. Boussaada O, Kamel MBH, Ammar S, Haouas D, Mighri Z, Helal AN. Insecticidal activity of some Asteraceae plant extracts against *Tribolium confusum*. Bulletin of Insectology 2008; 61(2):283-289.
4. Vinayaka KS, Kumar SVP, Kekuda PTR, Krishnamurthy YL, Mallikarjun N, Swathi D. Proximate composition, antioxidant, anthelmintic and insecticidal activity of a macrolichen *Ramalina conduplicans* Vain. (Ramalinaceae). Eur J Appl Sci 2009; 1(3):40-46.
5. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res 2012; 135(5):581–598.
6. Overgaard HJ, Sirisopa P, Mikolo B, Malterud KE, Wangensteen H, Zou Y, Paulsen BS, Massamba D, Duchon S, Corbel V, Chandre F. Insecticidal activities of bark, leaf and seed extracts of *Zanthoxylum heitzii* against the African malaria vector *Anopheles gambiae*. Molecules 2014; 19:21276-21290.
7. Mohammed BR, Abdulsalam YM, Deeni YY. Insecticide resistance to *Anopheles* spp. mosquitoes (Diptera: Culicidae) in Nigeria: A review. International Journal of Mosquito Research 2015; 2(3):56-63.
8. Dinesh DS, Kumari S, Pandit V, Kumar J, Kumari N, Kumar P, Hassan F, Kumar V, Das P. Insecticidal effect of plant extracts on *Phlebotomus argentipes* (Diptera: Psychodidae) in Bihar, India. Indian J Med Res 2015; 142:95-100.
9. Kumuda SS, Mohankumar TK, Prathibha KP, Vijayan VA. Efficacy of plant extracts against the larvae of filariasis vector, *Culex quinquefasciatus* Say and the dengue vector *Aedes aegypti* Linn at Mysore. Int J Curr Microbiol Appl Sci 2015; 4(6):242-249.
10. Hunter P. Challenges and options for disease vector control. EMBO Rep 2016; 17(10):1370-1373.
11. Hikal WM, Boeschen RS, Said-Al Ahl HAH. Botanical insecticide as simple extractives for pest control. Cogent Biol 2017; 31404274.
12. Venkadachalam R, Subramaniyan V, Palani M, Subramaniyan M, Srinivasan P, Raji M. Mosquito larvicidal and pupicidal activity of *Tephrosia purpurea* Linn. (Family: Fabaceae) and *Bacillus sphaericus* against, dengue vector, *Aedes aegypti*. Pharmacogn J 2017; 9(6):737-742.
13. Mbatchou VC, Tchouassi DP, Dickson RA, Annan K, Mensah AY, Amponsah IK, Jacob JW, Cheseto X, Habtemariam S, Torto B. Mosquito larvicidal activity of *Cassia tora* seed extract and its key anthraquinones aurantio-obtusin and obtusin. Parasites Vectors 2017; 10:562.

14. Sachin MB, Mahalakshmi SN, Kekuda PTR. Insecticidal efficacy of lichens and their metabolites- A mini review. J Appl Pharm Sci 2018; 8(10):159-164.
15. Okia M, Hoel DF, Kirunda J, Rwakimari JB, Mpeka B, Ambayo D, Price A, Oguttu DW, Okui AP, Govere J. Insecticide resistance status of the malaria mosquitoes: *Anopheles gambiae* and *Anopheles funestus* in eastern and northern Uganda. Malar J 2018; 17(1):157.
16. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4):564-582.
17. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali M, Siddiqui M, Khan AU. Antimicrobial activity of five herbal extracts against multidrug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules 2009; 14:586-597.
18. Al-Jiffri O, El-Sayed ZMF, Al-Sharif FM. Urinary tract infection with *Esherichia coli* and antibacterial activity of some plants extracts. Int J Microbiol Res 2011; 2(1):1-7.
19. Mishra MP, Padhy RN. In vitro antibacterial efficacy of 21 Indian timber-yielding plants against multidrug-resistant bacteria causing urinary tract infection. Osong Public Health Res Perspect 2013; 4(6):347-357.
20. Pavithra GM, Siddiqua S, Naik AS, Kekuda PTR, Vinayaka KS. Antioxidant and antimicrobial activity of flowers of *Wendlandia thyrsoidea*, *Olea dioica*, *Lagerstroemia speciosa* and *Bombax malabaricum*. J Appl Pharm Sci 2013; 3(6):114-120.
21. Ranjitha MC, Akarsh S, Kekuda PTR, Darshini SM, Vidya P. Antibacterial activity of some plants of Karnataka, India. J Pharmacogn Phytochem 2016; 5(4):95-99.
22. Jensen RH. Resistance in human pathogenic yeasts and filamentous fungi: prevalence, underlying molecular mechanisms and link to the use of antifungals in humans and the environment. Dan Med J 2016; 63(10): B5288.
23. Jensen RH, Hagen F, Astvad KM, Tyron A, Meis JF, Arendrup MC. Azole-resistant *Aspergillus fumigatus* in Denmark: a laboratory-based study on resistance mechanisms and genotypes. Clin Microbiol Infect 2016; 22(6):570.e1-570.e9.
24. Wiederhold NP. Antifungal resistance: current trends and future strategies to combat. Infect Drug Resist 2017; 10: 249-259.
25. Rupp S, Weber RW, Rieger D, Detzel P, Hahn M. Spread of *Botrytis cinerea* strains with multiple fungicide resistance in German horticulture. Front Microbiol 2017; 7:2075.
26. Sancheti A, Ju LK. Eco-friendly rhamnolipid based fungicides for protection of soybeans from *Phytophthora sojae*. Pest Manag Sci 2019; doi: 10.1002/ps.5418.
27. Chen J, Shen Y, Chen C, Wan C. Inhibition of key citrus postharvest fungal strains by plant extracts in vitro and in vivo: a review. Plants 2019; 8:26.
28. Gulcin I, Topal F, Sarikaya SBO, Bursal E, Bilsel G, Goren AC. Polyphenol contents and antioxidant properties of Medlar (*Mespilus germanica* L.). Rec Nat Prod 2011; 5(3):158-175.
29. Moukette BM, Pieme CA, Biapa PC, Njimou JR, Moor VJ, Stoller M, Bravi M, Ngogang JY. Phenolic content of *Hypodaphnis zenkeri* and its antioxidant effects against Fenton Reactions' mediated oxidative injuries on liver homogenate. Antioxidants (Basel) 2014; 3(4):866-889.
30. Moukette MB, Anatole CP, Biapa NCP, Njimou JR, Ngogang JY. Free radicals quenching potential, protective properties against oxidative mediated ion toxicity and HPLC phenolic profile of a Cameroonian spice: *Piper guineensis*. Toxicol Rep 2015; 2: 792-805.
31. Tan JB, Lim YY. Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts. Food Chem 2015; 172:814-822.
32. de Dicastillo LC, Bustos F, Valenzuela X, López-Carballo G, Vilariño JM, Galotto MJ. Chilean berry *Ugni molinae* Turcz. fruit and leaves extracts with interesting antioxidant, antimicrobial and tyrosinase inhibitory properties. Food Res Int 2017; 102:119-128.
33. Silva KDRR, Sirasa MSF. Antioxidant properties of selected fruit cultivars grown in Sri Lanka. Food Chem 2018; 238:203-208.
34. Kotresha K, Harihar NS. Uses of *Cochlospermum religiosum* (L.) Alston [Cochlospermaceae]: An ethnomedicinal plant. Indian Forester 2011; 137(3):393-394.
35. Yarra R, Aileni M, Kokkirala VR, Umate P, Vemunoori AK, Abbagani S. Micropropagation of *Cochlospermum religiosum* (L.) Alston. Tree and Forestry Science and Biotechnology 2011; 5:49-52.
36. Pandhure N, Gaikwad M, Waghmare V. In vitro tissue culture studies on *Cochlospermum religiosum* (Linn.). Trends Biotechnol Res 2012; 1(1):56-59.
37. Gahane RN, Kogje KK. Effect of pre-treatments for enhancing the germination of *Adansonia digitata* L. and *Cochlospermum religiosum* L. Indian Forester 2013; 139(7):648-651.
38. Rao SP, Neelima P, Lakshminarayana K, Kumar AO. Important plant-based non-timber forest products of west Godavari district, Andhra Pradesh, India. J Nat Prod Plant Resour 2014; 4(2):33-42.
39. Mishra T. Some potential folk herbal medicines for veterinary practices. European Journal of Pharmaceutical and Medical Research 2016; 3(8):347-352.
40. Khatoon S, Irshad S. Bark drugs as Indian ethnomedicine – Modern therapeutics and future prospects. In: Indian Ethnobotany: Emerging trends, Jain AK (Editor), Scientific Publisher, Jodhpur, India, 2016, Pp 87-98.
41. Chandrashekhar K. Critical review on notable resinous substance (Niryasa) used as botanical in Ayurveda. World Journal of Pharmaceutical and Medical Research 2018; 4(10):60-66.
42. Johnson-Fulton SB, Watson LE. Comparing medicinal uses of Cochlospermaceae throughout its geographic range with insights from molecular phylogenetics. Diversity 2018; 10: 123.
43. Patel RS, Chaudhari RP, Panchal P, Dalicha SB. Observation of some valuable trees with their medicinal uses and chemical properties from Gandhinagar, Gujarat, India . The World Journal of Engineering and Applied Science 2019; 5(1): 1-20.
44. Bhat GK. Flora of South Kanara. Akriti Prints, Mangalore, India, 2014.
45. Ankith GN, Rajesh MR, Karthik KN, Avinash HC, Kekuda PTR, Vinayaka KS. Antibacterial and antifungal activity of three *Ramalina* species. J Drug Delivery Ther 2017; 7(5):27-32.
46. Raghavendra HL, Kekuda PTR, Akarsh S, Ranjitha MC, Ashwini HS. Phytochemical analysis, antimicrobial and antioxidant activities of different parts of *Pleocaulus sessilis* (Nees) Bremek (Acanthaceae). Int J Green Pharm 2017; 11(2):98-107.
47. Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. J Vect Borne Dis 2007; 44:145–148.
48. Kweka EJ, Lyatuu EE, Mboya MA, Mwang'onde BJ, Mahande AM. Oviposition deterrence induced by *Ocimum kilimandscharicum* and *Ocimum suave* extracts to gravid *Anopheles gambiae* s.s (Diptera: Culicidae) in laboratory. J Glob Infect Dis 2010; 2(3):242–245.
49. Reegan AD, Gandhi MR, Paulraj MG, Ignacimuthu S. Ovicidal and oviposition deterrent activities of medicinal plant extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* Say mosquitoes (Diptera: Culicidae). Osong Public Health Res Perspect 2014; 6(1):64-69.
50. Benelli R, Zeller H, Van Bortel W. A review of the vector management methods to prevent and control outbreaks of West Nile virus infection and the challenge for Europe. Parasites Vectors 2014; 7:323.
51. Rawani A, Ghosh A, Chandra G. Mosquito larvicidal potential of four common medicinal plants of India. Indian J Med Res 2014; 140(1):102–108.
52. Mangalat S, Narayanan V, Janardhanan M. Herbal larvicides to control mosquito larvae, a preliminary study. Nat Prod Rad 2014; 3(1):24-26.
53. Perumalsamy H, Jang MJ, Kim JR, Kadarkarai M, Ahn JY. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. Parasites Vectors 2015; 8:237.
54. Benelli G, Jeffries CL, Walker T. Biological control of mosquito vectors: Past, present, and future. Insects 2016; 7:52.
55. Kim SI, Ahn YJ. Larvicidal activity of lignans and alkaloid identified in *Zanthoxylum piperitum* bark toward insecticide-susceptible and wild *Culex pipiens* pallens and *Aedes aegypti*. Parasites Vectors 2017; 10(1):221.
56. Abdallah EM. Plants: An alternative source for antimicrobials. J Appl Pharm Sci 2011; 1(6):16-20.
57. Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol 2012; 7(8): 979-990.
58. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. Antimicrobial resistance and the alternative resources with

special emphasis on plant-based antimicrobials-a review. Plants 2017; 6:16.
59. Panda SK, Mohanta YK, Padhi L, Park Y, Mohanta TK, Bae H. Large scale screening of ethnomedicinal plants for identification of potential antibacterial compounds. Molecules 2016; 21:293.
60. Zingare AK. Antimicrobial activity of *Adansonia digitata* and *Cochlospermum religiosum* extracts against *E. coli* and *S. aureus* isolates. International Journal of Researches in Biosciences, Agriculture and Technology 2015; Special Issue 6:11-14.
61. Goud SPP, Rama Murthy SK, Pullaiah T, Babu GVAK. Screening for antibacterial and antifungal activity of some medicinal plants of Nallamalais, Andhra Pradesh, India. J Econ Taxon Bot 2002; 26(3):677-684.
62. Bai JA, Rai RV, Samaga PV. Evaluation of the antimicrobial activity of three medicinal plants of South India. Malays J Microbiol 2011; 7(1):14-18.
63. Ponnamma P, Manasa G, Sudarshana MS, Murali M, Mahendra C. In vitro antioxidant, antibacterial and phytochemical screening of *Cochlospermum religiosum* (L.) Alston - A potent medicinal plant. Tropical Plant Research 2017; 4(1):13-19.
64. Kawde AB, Batra RJ, Weginwar RG, Akkewar DM, Gond GS, Aparna Y. Preliminary phytochemical screening and bioevaluatuion studies of stem bark of *Cochlospermum gossypium*. International Journal of Researches in Biosciences, Agriculture and Technology 2015; Special issue 1:199-206.
65. Pumpaluk P, Sritularak B, Likhitwitayawuid K, Lapirattanakul J. Antibacterial effect of herbal plants against three cariogenic microorganisms. M Dent J 2017; 37(1):71-80.
66. Anibal PC, de Cássia Orlandi Sardi J, Peixoto IT, de Carvalho Moraes JJ, Höfling JF. Conventional and alternative antifungal therapies to oral candidiasis. Braz J Microbiol 2010; 41(4):824-831.
67. Yoon MY, Cha B, Kim JC. Recent trends in studies on botanical fungicides in agriculture. Plant Pathol J 2013; 29(1):1-9.
68. Ngadze E. In vitro and greenhouse evaluation of botanical extracts for antifungal activity against *Phythopthora infestans*. J Biopest 2014; 7(2):199-204.
69. Ramaiah AK, Garampalli RH. In vitro antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*. Asian J Plant Sci Res 2015; 5(1):22-27.
70. Kekuda PTR, Akarsh S, Nawaz NAS, Ranjitha MC, Darshini SM, Vidya P. In vitro antifungal activity of some plants against *Bipolaris sorokiniana* (Sacc.) Shoem. Int J Curr Microbiol Appl Sci 2016; 5(6):331-337.
71. Soliman S, Alnajdy D, El-Keblawy AA, Mosa KA, Khoder G, Noreddin AM. Plants' natural products as alternative promising anti-Candida drugs. Pharmacogn Rev 2017; 11(22): 104-122.
72. Buch H, Arya A. Antifungal activity of selected plant extracts against three pathogenic fungi of *Gossypium herbaceum*. Current Research in Environmental and Applied Mycology 2017; 7(2):103–108.
73. Rajurkar NS, Hande SM. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian J Pharm Sci 2011; 73(2):146–151.
74. Kekuda PTR, Akarsh S, Darshini SM, Prafulla D, Raghavendra HL. Antiradical and antimicrobial activity of *Atylosia lineata* Wt. and Arn. Sci Technol Arts Res J 2015; 4(3):180-183.
75. Noreen H, Semmar N, Farman M, McCullagh JSO. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. Asian Pac J Trop Med 2017; 10(8):792-801.
76. Zhou JX, Braun MS, Wetterauer P, Wetterauer B, Wink M. Antioxidant, cytotoxic, and antimicrobial activities of *Glycyrrhiza glabra* L., *Paeonia lactiflora* Pall., and *Eriobotrya japonica* (Thunb.) Lindl. extracts. Medicines (Basel) 2019; 6(2):E43.
77. Hongsing P, Palanuvej C, Ruangrungsi N. Chemical compositions and biological activities of selected exudate gums. J Chem Pharm Res 2012; 4(9):4174-4180.
78. Ahmed AS, McGaw LJ, Elgorashi EE, Naidoo V, Eloff JN. Polarity of extracts and fractions of four *Combretum* (Combretaceae) species used to treat infections and gastrointestinal disorders in southern African traditional medicine has a major effect on different relevant in vitro activities. J Ethnopharmacol 2014; 154(2): 339-350.
79. Sandeepa KH, Harsha TS, Prashanth M, Raghavendra HL. In vitro antioxidant activity of *Anaphalis lawii* (Hook. f) Gamble and *Helichrysum buddleioides* DC - a comparative study. J Biosci Agric Res 2017; 12(2):1064-1073.
80. Chung Y, Chien C, Teng K, Chou S. Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb & zucc. Food Chem 2006; 97: 418-425.
81. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. Ayu 2013; 34(2):209-214.
82. Nariya PB, Bhalodia NR, Shukla VJ, Acharya R, Nariya MB. In vitro evaluation of antioxidant activity of *Cordia dichotoma* (Forst f.) bark. Ayu 2013; 34(1):124-128.
83. Ravishankar K, Kiranmayi GVN, Prasad RY. Comparative in vitro antioxidant activities of ethanolic extract, ethyl acetate extract (EAE), and hexane extracts (HE) of *Tecoma gaudichaudi* flowers. Int J Green Pharm 2018; 12(S1):S214-S219.
84. Leterme P, Buldgen A, Estrada F, Londono AM. Mineral content of tropical fruits and unconventional foods of the Andes and the rain forest of Colombia. Food Chem 2006; 95: 644-652.
85. Dileep N, Rakesh KN, Junaid S, Kumar RKA, Kekuda PTR, Vijayananda BN. Elemental analysis, anticariogenic, insecticidal and anthelmintic activity of *Anaphalis lawii* (Hook.f.) Gamble. Res J Pharm Tech 2013; 6(5): 569-574.
86. Sadia H, Ahmad M, Sultana S, Abdullah AZ, Teong LK, Zafari M, Bano A. Nutrient and mineral assessment of edible wild fig and mulberry fruits. Fruits 2014; 69(2):159-166.
87. Cristina HMR, Gabriel HM, Petru N, Radu S, Adina N, Ducu S. The monitoring of mineral elements content in fruit purchased in supermarkets and food markets in Timisoara, Romania. Annals of Agricultural and Environmental Medicine 2014; 21(1): 98–105.
88. Sudhakaran A, Nair GA. Nutritional evaluation of fruits of *Gynochthodes umbellata* (L.) Razafim. & B. Bremer–An underutilized edible fruit plant. Pharmacogn J 2016; 8(1):72-76.