Shobuj Bangladesh Publications House
International Journal of Natural and Social Sciences
ISSN: 2313-4461

Evaluation of some medicinal plants against *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*

Nigar Sultana Meghla[1], Maruf Hossain[1], Badrul Alam[1], Lovely Rani Paul[1], Nigarin Sultana[1], Ashish Kumar Das[2], Md. Bakhtiar Lijon[2]*

[1]Department of Microbiology, Jessore University of Science and Technology, Jessore-7408, Bangladesh
[2]Modern Food Testing Laboratory, Chittagong City Corporation, Chittagong, Bangladesh

ABSTRACT

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value and even in modern times have formed the basis of many pharmaceuticals in use. The aim of the present study was to evaluate the antimicrobial activity of different extracts of *Piper betle, Boerhavia diffusa, Oxalis corniculata, Centella asiatica, Camellia sinensis, Curcuma longa and Allium cepa* traditionally used in Bangladesh. The antimicrobial activity of these different solvent extracts were tested against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacterial species (*Escherichia coli* and *Salmonella* spp.) by observing the zone of inhibition by disc diffusion method. The tested bacterial species were isolated from hospital waste water sample and fresh poultry meat samples where Chloramphenicol was used as standard for antibacterial assay. The results of the study revealed that the ethanolic, methanolic, and aqueous extracts of almost all the seven herbal species successfully showed to be effective against the tested Gram-positive and Gram-negative bacteria with only negative result observed in case of *Centella asiatica.* The methanolic and water extract of *Boerhavia diffusa* was found to be slightly effective producing a shorter zone of inhibition. Among these medicinal plant products, *Camellia sinensis* and *Piper betle* was found to be more effective against the tested bacterial species where methanolic extracts showed maximum zone of inhibition against Gram-positive (*S. aureus* with zone of inhibition of 12 mm and 11 mm respectively) and Gram-negative bacteria (e.g. *Salmonella* spp. with zone of inhibition of 16 mm and 14 mm). The result confirmed the presence of antibacterial activity of *Piper betle* extract and other five medicinal plant extracts against various human pathogenic bacteria which should be further tested for other pathogenic multi drug resistant bacteria and could be used as an effective biocontrol agent in future.

Keywords: Medicinal plants, antibacterial property, Gram positive bacterium, Gram negative bacterium.

*Corresponding author.
E-mail address: lijonmicro2014@gmail.com (MB Lijon)

INTRODUCTION

Although antibiotics are considered to save the lives of millions of people, the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant (MDR) pathogens (Bandow et al., 2003). The promiscuous use of antibiotics accounts for a major part of the community burden of antibiotic use and contributes dramatically to the rising prevalence of resistance among major human pathogens. The resistance problem demands that a renewed effort be made to screen various medicinal plants for their potential antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. So it is necessary to identify newer antibiotics from herbal sources which are devoid of such serious side effects (WHO, 1999). Plants have the most important bioactive compounds including alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids and gums which are capable of producing definite physiological action on body. Medicinal plants are relied upon by 80% of the world's population (Hashim et al., 2010) and in Bangladesh there is a rich tradition of using herbal

How to cite this article: Meghla NS, Hossain M, Alam B, Paul LR, Sultana N, Das AK and Lijon MB (2016). Evaluation of some medicinal plants against *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*. International Journal of Natural and Social Sciences, 3(1): 25-31.

medicine for the treatment of various infectious diseases, inflammations, injuries and other diseases. Many of the plant materials used in traditional medicine are generally proved to be more effective and relatively cheaper than modern medicine (Mann et al., 2008). The present study was undertaken to evaluate the seven different medicinal plant extracts of *Boerhavia diffusa, Piper betel, Oxalis corniculata, Centella asiatica, Camellia sinensis, Curcuma longa and Allium cepa* against Gram-negative bacteria; *Escherichia coli*, *Salmonella* spp. and Gram-positive bacterium, *Staphylococcus aureus*.

Piper betel L. belongs to the family Piperaceae. This family usually contains herbs or shrubs often with swollen nodes. The essential oil from the leaves is used in the treatment of catarrhal disorders and as an antiseptic. A preliminary study reported that *Piper betl*e leaves extracts contains large numbers of bioactive molecules like polyphenols, alkaloids, steroids, saponins and tannins (Koff et al., 1971). *Boerhavia diffusa* L. (Nyctaginaceae) is a perennial herbaceous plant growing in tropical regions such as the Antilles, South America, India and Africa. It contains about 0.04 % of alkaloids known as punarnavine and punernavoside, an antifibrinolytic agent. It also contains about 6% of potassium nitrate, an oily substance, and ursolic acid (Kokate et al., 2005). In *Boerhavia diffusa*, maximum inhibition was observed in *Staphylococcus aureus* followed by *Bacillus megaterium* and *Bacillus cereus* respectively at 50 µL of concentration (Girish and Satish, 2008).

Oxalis corniculata Linn. is a small procumbent herb, with stems rooting and pubescent with appressed hairs,leaves palmately 3-foliolate, widely used against numerous infectious diseases and also known to cure dysentery, diarrhea and skin diseases (Kirtikar and Basu, 1975). *Centella asiatica* (L) urban belonging to the family Umbeliferae is a common perennial herbaceous creeper flourishing abundantly in moist areas. It is claimed to possess a wide range of pharmacological effects, being used for human wound healing, mental and neurological disorders, atherosclerosis, fungicidal, antibacterial, antioxidant and anticancer purposes. *C. asiatica* has been also reported to be useful in the treatment of inflammations, diarrhea, asthma, tuberculosis and various skin lesions and ailments like leprosy, lupus, psoriasis and keloid (Ullah etn al., 2009).

Camellia sinensis is a species of evergreen shrub or small tree whose leaves and leaf buds are used to produce tea. The leaves have been used in traditional Chinese medicine and other medical systems to treat asthma (functioning as a bronchodilator), angina pectoris, peripheral vascular disease, and coronary artery disease. Among other interesting bioactivities, (-)-catechin from *C. sinensis* was shown to act as agonist of PPARgamma, nuclear receptor that is current pharmacological target for the treatment of diabetes type-2 (Wang et al., 2014). Turmeric (*Curcuma longa* L.) belongs to the family Zingiberaceae, is a perennial rhizomatous shrub native to Southern Asia. *The* extract of it contains alkaloids, tannin, flavonoid, glycoside and carbohydrate. There are reports showing that alkaloids and flavonoids are the responsible compounds for the antibacterial activities in higher plants (Cordell et al., 2001).

Allium is the largest and important representative genus of the *Alliacae* family comprises 450 species. Onion *(Allium cepa)* is a bulbous plant widely cultivated in almost every country of the world with leading production in China, India and United States. It is rich in proteins, carbohydrates, sodium, potassium and phosphorus. Traditionally onion has been used to treat intestinal infections. It has been reported to be an antibacterial, antiviral, antiparasitic, antifungal and has antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti inflammatory and antioxidant activity (Lampe, 1999).

MATERIALS AND METHODS

Processing of medicinal plants

Piper betle, Boerhavia diffusa, Oxalis corniculata, Centella asiatica, Camellia sinensis, Curcuma longa and Allium cepa were purchased from local shops, washed twice with tap water followed by 95% ethanol and then with distilled water. The leaves and seeds were then air-dried under shaded sunshine and grinded to make fine powder. Course materials were sieved out and re-grinded.

Extraction of medicinal plants

The conical flasks were sterilized in the oven 160˚C for 30 minutes. Five (5) gm of powder from each of the samples was poured into the conical flask. In case of methanolic extract, 50 mL of 100% methanol was added to each flask and mixed it perfectly. In case of ethanolic extract, 50 mL of 100% ethanol and to prepare water extract, 50 mL of distilled water was used and then incubated it in the incubator for seven days at 28 ˚C. The extracts were filtered out with filter paper cone and put the supernatant in each petriplate and removed the debris from the conical flask. The plates containing extracts were dried at 45 ˚C. Weight of each of the extracts was calculated following the calculation (W=W3-W2) showed in Table 1. The tubes were then dried until it came to the point of 0.5 mL and covered with cap.

Table 1
Representative measurement of the respective solvent extract.

Respective herbal products	Weight of blank microcentrifuge tube (W1)	Weight of water/methanol/ ethanol and tube (W2)	Weight of water/methanol/ethanol/ tube and extract (W3)	Weight of extract W=W3-W2

Disc preparation

Whatman filter paper was punched by punch machine into 6mm in diameter. Then these disc papers were autoclaved to be sterilized and dried. All the extracts were imprignated into the discs (10 mg/disc) and tested against a number of bacterial pathogen.

Preparation of inoculum for disc diffusion assay

The stock culture of human pathogenic bacteria (*E. coli, Salmonella* spp. and *Staphylococcus aureus*) were revived from glycerol broth stock to Nutrient agar media followed by the selective growth onto EMB, XLD and Mannitol Salt Agar media respectively and then inoculated to nutrient broth (one loop full culture in 5mL nutrient broth). After incubation at 37 ˚C for 2 hrs until confirmation of the McFarland standard and then culture from broth were swabed on the Mueller Hinton Agar plate and implanted the standard antibiotic disc as the control along with the prepared discs (soaked with respective extracts) into the plates. The Mueller Hinton Agar plates were incubated at 37 ˚C for 24 to 48 hrs and observed the zone of growth inhibition (Figure 1).

RESULTS AND DISCUSSION

Here figure 2 shows the effects of all the six herbal plant species where the bacterial strains were more susceptible to *Camellia sinensis* and *Piper betle.*

In the present study, methanolic, ethanolic and water extracts of seven medicinal plants namely *Piper betle*, *Boerhavia diffusa, Oxalis corniculata, Centella asiatica, Camellia sinensis, Curcuma longa and Allium cepa* were screened against human pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. by disc diffusion method (Alzoreky and Nakahara, 2003). Similar methods were used by Ayman and Mazen (2014) and Nayan et al., (2011). Out of these seven herbal plants methanolic, ethanolic and water extract of *Piper betle*, *Oxalis corniculata, Camellia sinensis, Curcuma longa* showed to be most effective for these bacterial species. The present study is in agreement with the findings of Datta et al., (2011) who reported slightly effectiveness of *Boerhavia diffusa* and *Centella asiatica,* no zone of inhibition for *Camellia sinensis and* strongest zone of inhibition for *Piper betle* extract against the tested bacterial species. Similar findings also reported by Orafidiya et al., (2000) where Methanolic extract of *Camellia sinensis* and *Piper betle* showed the maximum zone of inhibition of 16 mm and 14 mm respectively against *Salmonella* spp. followed by a zone of 11 mm and 12 mm against *Escherichia coli* and 12 mm and 11 mm against *Staphylococcus aureus*.

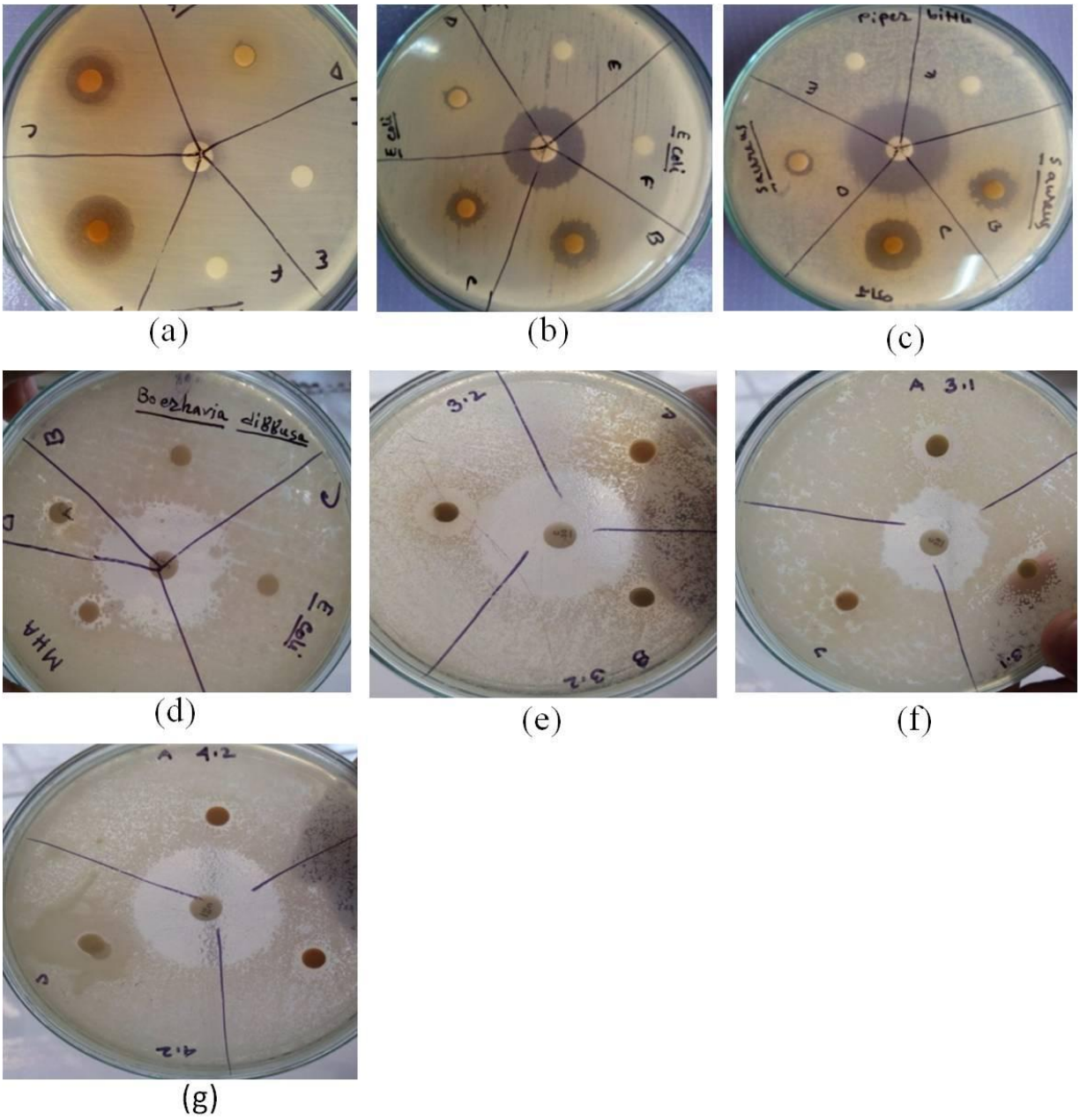

Figure 1
Pictorial representation of zone of inhibition of (a) *Piper betle* (b) *Boerhavia diffusa* (c) *Oxalis corniculata* (d) *Camellia sinensis* (e) *Curcuma longa* (f) *Allium cepa* and (g) *Centella asiatica* against three types of bacterial species on mueller hinton agar plates.

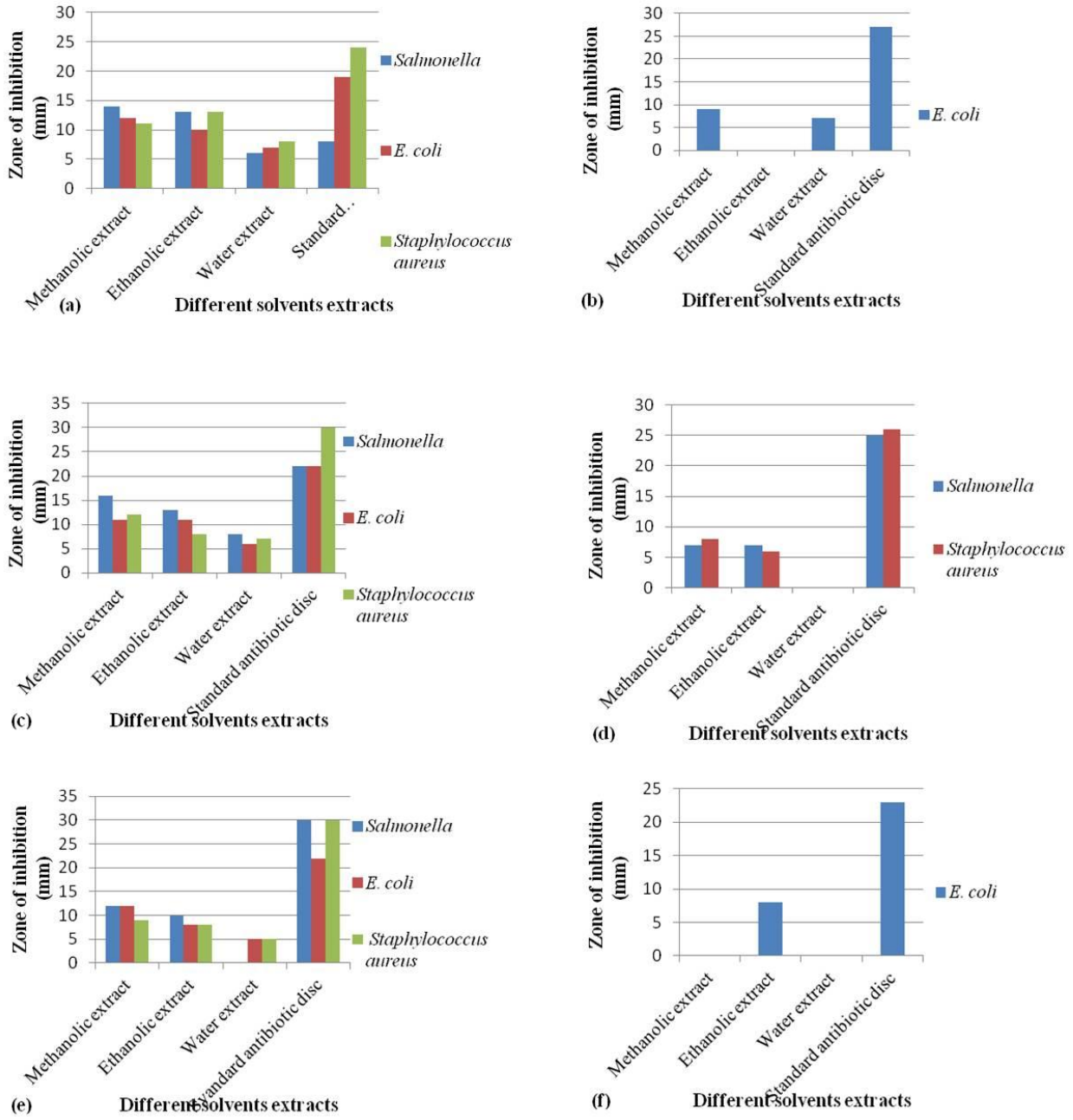

Figure 2
Graphical representation of different types of solvent extracts (a) *Piper betle* (b) *Boerhavia diffusa* (c) *Camellia sinensis* (d) *Oxalis corniculata* (e) *Curcuma longa* (f) *Allium cepa* against respective bacterial species.

Ethanolic extracts of these two plants showed maximum zone of inhibition of 8mm and 13mm respectively against *Staphylococcus aureus* followed by a zone of 13 mm and 12 mm against *Salmonella* spp. and 11 mm against *Escherichia coli*. Water extracts showed maximum zone of inhibition of 7 mm and 8mm against *Staphylococcus aureus* followed by a zone of 6 mm and 7 mm against *Escherichia coli* and 8 mm and 6mm against *Salmonella* species. Taley et al., (2012) investigated the methanol and aqueous extracts of *O. corniculata* leaves for antibacterial activities against some of the bacterial strains namely *E. coli*, *S. aureus*, *P. aeruginosa*, *P. vulgaris*, and *B. subtilis* where

they reported the zone of inhibition in the range of 6–14 mm against these pathogens among which *B. subtilis* showed maximum zone of inhibition (14 mm)while, in the present investigation, different solvents extracts of *O. corniculata* were evaluated for antibacterial activities and obtained significant results although *Boerhavia diffusa* and *Centella asiatica* were slightly effective, with less susceptibility for the bacterial species, and *Camellia sinensis* showed no zone of inhibition for these selected bacteria.

The results in this study indicated that almost all the selected medicinal plants extract was more effective to the particular microorganism which agrees with the findings of Orafidiya et al., (2000) where antibacterial activity was screened against Gram negative bacteria. For the present study it is suggested that *Piper betle* plant leaves along with other five medicinal plant species may be recommended as useful sources to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost-effective.

CONCLUSION

It can be concluded that the leaves of *Piper betel* along with *Oxalis corniculata, Centella asiatica, Curcuma longa* and *Allium cepa* can be very good source for herbal drugs at any solvents used in this study. The study deserve more extensively work on isolation and identification of active ingredients from the tested plants having antibacterial properties in order to explore its potential in the treatment of many infectious diseases.

REFERENCES

Alzoreky NS and Nakahara K (2008). Antibacterial activity of extracts from some edible plants commonly consumed in Asia, International Journal of Food Microbiology, 80:223–30.

Ayman Al-Mariri and Mazen Safi (2014). In vitro antibacterial activity of several plants extracts and oils against some Gram negative bacteria, Iranian Journal of Medical Science, 39: 36-43.

Bandow JE, Brötz H, Leichert LI, Labischinski H and Hecker M (2003). Proteomic approach to understanding antibiotic action, Antimicrobial Agents Chemotherapy, 47:948–55.

Cordell GA, Quinn-Beattie ML and Farnsworth NR (2001). The potential of alkaloids in drug discovery, Phytotherapy Research, 15: 183–205

Datta A, Ghoshdastidar S and Singh M (2011). Antimicrobial property of *Piper betel* leaf against clinical isolates of bacteria, International Journal of Pharmaceutical Sciences and Research, 2:104-109

Girish HV and Satish S (2008). Antibacterial Activity of Important Medicinal Plants on Human Pathogenic Bacteria- a Comparative Analysis, World Applied Sciences Journal, 5: 267-271.

Hashim H, Kamali EL and Mohammed Y (2010). Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants, Current Research Journal of Biological Science, 2: 143-146.

Kirtikar Basu (1975). Indian medicinal plants. 3rd Edition, Volume- I, 437, M.S. periodical experts New Delhi-32.

Koff RS, Gordan G and Sabesin SM (1971). D-galactosamine hepatitis hepatocellular injury and fatty liver are following a single dose, Proceedings in Society of Experimental Biology and Medcine, 137: 696-701.

Kokate CK, Purohit AP and Gokhale SB (2005). Textbook of Pharmacognosy. Thirty eight Edition, NiraliPrakashan, Pune, 537-538.

Lampe JW (1999).Health effects of vegetables and fruits: assessing mechanisms of action in human experimental studies, American Journal of Clinical Nutrition, 70:475–90.

Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K and Oladosu P (2008). Evaluation of *in vitro* anti mycobacterial activity of Nigerian plants used for treatment of respiratory diseases, African Journal of Biotechnology, 7:1630–6.

Nayan R, Bhalodia and Shukla VJ (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* I.: an ethanomedicinal plant. Journal of Advanced Pharmaceutical Technology and Research, 2: 104–109.

Orafidiya OO, Eluyoba AA, Iwalewa FO and Okeke IN (2000). Evaluation of antidiarrhoea properties of *Ocimum gratissimum* volatile oil and its activity against enteroaggregative *Escherichia coli*, Pharmaceutical and Pharmacological Letters, 10: 9-12.

Taley SL, Pethe AS and Rothe SP (2012). Studies on antibacterial activity of some plant extracts, International Multidisciplinary Research Journal, 2: 17–18.

Ullah MO, Sultana S and Haque A (2009). Antimicrobial, cytotoxic and antioxidant activity of *Centella asiatica*, European Journal of Scientific Research, 30:260-264.

Wang L, Waltenberger B, Pferschy-Wenzig EM, Blunder M, Liu X, Malainer C, Blazevic T, Schwaiger S, Rollinger JM, Heiss EH, Schuster D, Kopp B, Bauer R, Stuppner H, Dirsch VM and Atanasov AG (2014). Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review, Biochemical Pharmacology, pii: S0006-2952(14)00424-9.

WHO (1999).WHO Monographs on selected medicinal plants, 1:16-32.