The Pharma Innovation Journal 2019; 8(1): 542-547

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(1): 542-547

www.thepharmajournal.com
Received: 03-11-2018
Accepted: 07-12-2018

S Premalatha
PG & Research, Department of Botany, Government Arts College Autonomous, Coimbatore. Tamil Nadu, India

G Ramar
PG & Research, Department of Botany, Government Arts College Autonomous, Coimbatore. Tamil Nadu, India

Correspondence
S Premalatha
PG & Research, Department of Botany, Government Arts College Autonomous, Coimbatore. Tamil Nadu, India

Anti-bacterial properties of fractions isolated from *Couroupita guianensis* and *Atalantia monophylla*

S Premalatha and G Ramar

Abstract
In the present study different fractions from ethanol extracts of *Couroupita guianensis* and *Atalantia monophylla* were isolated and tested for their antibacterial bioassay on selected pathogens. Antibacterial activity was determined against three gram positive bacteria *Staphylococcus aureus*, *Streptococcus epidermidis*, *Bacillus subtilius* and four Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae*, *Proteus vulgaris* was carried in well diffusion method. The results obtained fractions from ethanol crude extracts of *Couroupita guianensis* and *Atalantia monophylla* had inhibitory effect on the growth of isolates. The effect exhibited by ethanolic extract fractions was significantly greater than the fraction II and fraction III. Gram negative bacteria *Escherichia coli* and the Gram positive bacteria *Staphylococcus aureus* showed the highest zone of inhibition in ethanol extracts fractions II and III of *Couroupita guianensis* and *Atalantia monophylla*.

Keywords: Anti-bacterial activity, *Couroupita guianensis*, *Atalantia monophylla*, *Staphylococcus aureus*

Introduction
Plants play a major role in our ecosystem. By nature we have abundant medicinal plants in the world balancing life in the ecosystem. Indians used food as medicine and vice versa, but after few decades food became one of the source to cause the diseases. Ancestral methods are still being followed to an extent but the plants of medicinal value are forgotten over a period of time. Our ancestors and various tribes in the world were living alongside with these medicinal plants keeping their health in good condition by using the right plants to cure diseases of different nature. Some methods and treatments are still remembered but we have to accept the fact that the value of most medicinal plants are long forgotten as they are not scripted anywhere. Newer generation are in the hunt to research on these medical plants to extract medicines to cure newly arising diseases caused by micro-organisms day by day. Though advanced medicines have been found to cure various diseases, we are still unable to stop the death rate due to diseases caused by micro-organisms. So most developed and under-developed nations have turned towards these medicinal plants and started spending more on the research works to find cure for these diseases which is the right direction to go. E.g., Recently, Nilavembu Kashayam (Nilevembu in liquid form) has been found as a source to cure severe viral fevers in India especially in the state of Tamil Nadu. Plant based medicines always prove to be cheaper with less side effects. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase of new and re-emerging infectious due to variety of bacterial agents. The natural products such as plant extracts provide unlimited opportunities for new drug discoveries, mostly because of excessive of varieties of phytochemicals (Sasidharan *et al.*, 2011) [1].

Thus natural products have been a major source of drugs for centuries. *Couroupita guianensis* Aubl is belonging to family Lecythidaceae and its found extensively all over the Indian subcontinent and North-eastern South Africa. The plant has regional synonyms such as cannon ball tree, Shivalingam and Kailashpati in English, Kannada and Hindi respectively (Sumathi and Anuradha 2017) [2]. In India, this tree is sacred to Hindus, who believe its hooded flowers look like the Naga, it is found in Thanjayur big temple and it is grown at most of the Shiva temples in Tamil Nadu.

Atalantia monophylla Linn belongs to the family Rutaceae, comprises of 11 species which are closely-related. The leaves are used for the treatment of snakebite by local Malayali tribes of Kolli hills.

As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is a need to develop alternative antimicrobial drugs. One of the approach is to screening the local medicinal plants which represent a rich source of novel antimicrobial agents (Reddy *et al*., 2010) [19]. The present study aimed to analyse the antibacterial properties of fractions from *Couroupita guianensis* and *Atalantia monophylla*.

Materials and Methods

Collection of plant Materials

The leaves of *Couroupita guianensis* and *Atalantia monophylla* was collected from in and around Tiruchirapalli District, Tamil Nadu, India. Plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India. The voucher specimen of *Couroupita quinensis* and *Atalantia monophylla* was prepared and deposited in PG and Research Department of Botany, Government Arts College, Coimbatore, Tamil Nadu, India.

Extraction of Plant material

The plants leaves were thoroughly washed with tap water and shade dried under room temperature (27.0 ± 2ºC and 75 ± 5% RH). After complete drying the leaves was powdered using electric blender and sieved through a kitchen strainer. 1000g of plant powder was extracted by soxhlet extraction (Plate I) methods with ethanol of solvents and filtered through What Man's No. 1 filter paper. The solvent from the crude extract were condensed with Rotary Vacuum Evaporator (Plate II) and condensed extracts were collected in clean borosil vials (Plate II) and stored in the refrigerator.

Thin Layer Chromatography (TLC)

Thin layer chromatography was done on pre-coated plates Silica Gel G (0.2mm thick; Merck, India) were trimmed with strips and the position of the origin marked by a straight line. Ethanol extracts of *Couroupita guianensis* and *Atalantia monophylla* and their fractions were dissolved in hexane and were spotted on the plate with fine capillary tube at the height of 0.8-1.0cm from the base. In the present study, different solvent mixtures *viz*; hexane and ethanol, were used for developing the TLC plates to get better results. After putting the plates in solvent system, the appropriate distance moved by the solvent was measured to find the retention factor (nearly 2/3 of plate). The plates were air dried and developed with iodine in iodine chamber, under UV light nm254 and nm365 and spraying the TLC plates with 10% sulphuric acid. The Retention Factor (Rf) values of all compounds isolated were calculated.

$$\mathrm{Rf} = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the solvent front}}$$

Column Chromatography (CC)

The ethanol extracts of *Couroupita guianensis* and *Atalantia monophylla* for the isolation of fractions used by Column chromatography. Silica gel -100 - 200 mesh (Molychem) was packed in a column (size 60cm x 4 cm) with hexane using the wet slurry method. This involves preparing a solution of silica gel, with hexane in this case, in a beaker and subsequently adding this unto the column till it is about three fourths filled. The solution was stirred for dispersal and quickly added to the column before the gel settles. This method was used to prevent the trapping of air bubbles. A ball of wool was pushed into the column to settle a top the packed silica gel. For the elucidation of components, the polarity of the solvent (mobile phase) was increased using a combinations of hexane, hexane: chloroform (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9), then chloroform and Similarly the column was run over chloroform, then chloroform: ethanol (9:1, 8:2 and 2:8) and then ethanol respectively. The volume of the collected fractions was 10ml each test tubes. Totally 300 fractions of *Couroupita guianensis* and *Atalantia monophylla* were collected. TLC plate was run on every column fraction and it was exposed to iodine vapour to investigate the spots to calculate Rf values. Fractions in which similar spots appeared were collected in one pool. The fractions with similar Rf values were pooled and isolates designated as three fractions for *Couroupita guianensis* and *Atalantia monophylla* were obtained. All the isolated fractions were stored in solid form for further experimentation.

Test microorganisms

The test organisms used were clinical isolates *viz Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilius, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae* and Proteus vulgaris the bacterial cultures were maintained on Muller Hinton agar medium. Standard antibiotics of Gentamycine for *S. aureus, S. epidermids Bacillus subtilius, E. coli P. aeruginosa, S. typhi, V. cholerae* and *P. vulgaris* was used as reference control.

Anti-bacterial Activity

The fractions from ethanol extracts of *Couroupita guianensis* and *Atalantia monophylla* with three different concentrations were tested for antibacterial activity using agar disc diffusion assay method (Chung *et al*., 1990) [3].

Inoculum Preparation

Inoculums of each bacterial strain was suspended in 10 ml Muller-Hinton agar medium and incubated overnight at 37°c cultures will be diluted/10 with Muller-Hinton broth before use.

Disc-Diffusion Method

Antibacterial activity will be demonstrated using a modification of the method originally described by Bauer *et al.,* (1966) [4] which is widely used for the antibacterial susceptibility testing (Barry and Thornsberry, 1985) [5].

Result

Considering the evolution of resistance genes to antibiotics of microbial origin and non-antibiotic chemicals (Lee *et al*., 2003) [6], plant materials have become the subject of public attention and therefore the pharmaceutical industry is moving away from drug discovery or screening towards compounds isolated by medicinal plants. The different fractions from ethanol crude extracts plants *Couroupita guianensis* and *Atalantia monophylla* leaves were evaluated for their antibacterial bioassay on selected pathogens and results are tabulated 1 & 2.

The plants *Couroupita guianensis* and *Atalantia monophylla* leaves fractions were evaluated for their antibacterial activity against nine clinical bacterial isolates namely *Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilius,* three

Gram positive bacteria and *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera and Proteus vulgaris six* Gram negative bacteria. The screening of the three fractions reveals that, fraction I, II, and III and these fractions were subjected to disc diffusion assay against the bacterial pathogens the zones of inhibition obtained for the various micro-organisms are showing remarkable activity against the micro-organisms tested.

The data pertaining to the above experiments are shown in table 1. Fraction I, II, III are promising in controlling the growth of bacterial strain in the nutrient agar (Muller Hilton) medium and their values ranging from 9-39 zone of inhibition (mm) in *Couroupita guianensis* leaves fractions I, II, III were performed at 10 µl/ml, 20 µl/ml, and 30 µl/ml concentration. Besides, fraction I. showing least activity against the *Vibrio cholera* 9mm zone of inhibition at 10 µl/ml concentration, 15mm in 20 µl/ml and 19mm at 30 µl/ml concentration *and Klebsiella pneumoniae* 10mm at 10 µl/ml, 15mm and 20 mm at 30 µl/ml concentration. While the maximum values of zone of inhibition are recorded from *Staphylococcus aureus* and *Escherichia coli* bacteria, 29 mm and 28 mm at 30 µl/ml concentration from fraction I respectively.

The *Couroupita guianensis* leaves fractions II, showed potent activity against all the organisms tested and minimum values of zone of inhibition showed 13mm zone of inhibition at 10 µl/ml, 17mm at 20 µl/ml and 19mm at 30 µl/ml. And the maximum values of zone of inhibition are recorded from *Staphylococcus aureus* 25mm at 10 µl/ml, 33mm at 20 µl/ml and 39mm at 30 µl/ml, *Bacillus subtilius*19mm at 10 µl/ml, 23mm at 20 µl/ml and 37mm at 30 µl/ml and *Escherichia coli* bacteria, 19mm and 21 mm and 35mm at 10, 20, 30 µl/ml concentration from fraction II were recorded.

Similarly fraction III showed 9 mm zone of inhibition against *Vibrio cholerae*13 mm at 30 µl/ml followed by *Salmonella typhi* 15mm *and Pseudomonas aeruginosa* 19mm 30 µl/ml. The maximum zone of inhibition was noticed in *Staphylococcus aureus* 15mm at 10 µl/ml, 21mm at 20 µl/ml and 31mm at 30 µl/ml, *Bacillus subtilius*13mm at 10 µl/ml, 19mm at 20 µl/ml and 29mm at 30 µl/ml and *Escherichia coli* bacteria, 15mm at 10 µl/ml, 17mm at 20 µl/ml and 27mm at 30 µl/ml.

Plant extracts and compounds are of new interest as antimicrobial agents. As a result, the antimicrobial activity of *Atalantia monophylla* plant leaves fractions was screened against the most common pathogens such as Gram positive (*Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilius) and Gram negative* (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Proteus vulgaris*). The activity was measured in terms of zone of growth inhibition in mm. It is interesting to note that *Klebsiella pneumoniae*11mm at 10 µl/ml, 15mm at 20 µl/ml and 19mm zone of inhibition at 30 µl/ml, *Escherichia coli* showed 12mm at 10 µl/ml, 15mm at 20 µl/ml and 19mm zone of inhibition at 30 µl/ml and in the case of *Staphylococcus aureus*, 9mm at 10 µl/ml, 13mm at 20 µl/ml and 17mm zone of inhibition at 30 µl/ml of the *Atalantia monophylla* plant leaves fractions I. The *Proteus vulgaris* 7mm at 10 µl/ml, 11mm at 20 µl/ml and 12mm zone of inhibition at 30 µl/ml are recorded as the minimal concentration which inhibits the growth of the bacteria. Whereas, not high but moderate activity is obtained from the plants of *Couroupita guianensis* and *Atalantia monophylla* leaves fractions against the Gram-negative *Pseudomonas aeruginosa, Salmonella typhi* and *Proteus vulgaris* bacteria.

The maximum zone of inhibition clearly seen in *Klebsiella pneumonia, Staphylococcus, aureus, Escherichia coli* at 28mm, 26mm and 26mm at 30 µl/ml of fraction II and the similar trends are also reflected in fraction III. *Escherichia coli* 14mm at 10 µl/ml, 17mm at 20 µl/ml and 21mm zone of inhibition at 30 µl/ml followed by *Klebsiella pneumoniae* 10mm at 10 µl/ml, 14mm at 20 µl/ml and 19mm zone of inhibition at 30 µl/ml, *Staphylococcus aureus* shows highly sensitive at 14mm at 10 µl/ml, 19mm at 20 µl/ml and 21mm zone of inhibition at 30 µl/ml of the *Atalantia monophylla* plant leaves fraction III respectively. Among the three fractions, fraction II shows promising bacterial growth inhibition activity in both *Couroupita guianensis* and *Atalantia monophylla* against all the species.

Discussion

Some of these metabolites particularly some flavonoids that were detected were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Yoshi's *et al.,* 2008). In addition, some alkaloids and tannins are well documented for antimicrobial activity (Tringali C, 2005; Mansouri S *et al*., 2001) [9, 10]. We conducted prior research on *Couroupita guianensis* Preliminary phytochemical and the antimicrobial activity in Dichloromethane, Chloroform and Ethyl acetate extracts. Among the crude extracts tested, Dichloromethane extract showed the presence of protein, terpenoinds tannins and ethyl acetate extract showed the presence of alkaloid, flavonoid, and carbohydrate and saponin compounds. Antibacterial activity of Dichloromethane, Chloroform and Ethyl acetate extracts of *Couroupita guianensis* tested against five important human pathogenic bacteria *viz., Salmonella typhi, Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella nemoniae* at 25, 50, 75 and 100µl concentrations and the data pertaining to the experiments are shown in table 2. Among the tested bacteria the results showed the highest zone of inhibition against *Staphylococcus aureus* is 23 mm in 100 µl /ml Dichloromethane extract and *Escherichia coli* also showed 23 mm in 100 µl /ml chloroform extract. Whereas, ethyl acetate extracts showed the maximum zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* is 21mm in 100 µl /ml (Karthi and Premalatha, conference proceedings, 2017) [18].

In our previous studies, preliminary phytochemical and antibacterial studies of Ethanol, Chloroform and Ethyl acetate extract obtained from the leaves of *Atalantia monophylla* for its medicinal potentials. The effect exhibited by ethanol extract of leaves was significantly greater than the Chloroform and Ethyl acetate leaves extracts. Gram negative bacteria Escherichia coli and the Gram positive bacteria *Staphylococcus aureus* showed the highest zone of inhibition in all the three ethanol, chloroform and Ethyl acetate extracts. The phytochemical screening revealed the presence Carbohydrates, alkaloids, flavonoids, Cardiac glycosides, Protein and Phenolic compounds found in *Atalantia monophylla* ethanol leaves extracts. The experimental data clearly revealed that the effect exhibited by ethanol extract of leaves was significantly greater than the Chloroform and Ethyl acetate leaves extracts (Premalatha and Karthi, 2017) [18].

The purpose of this study was to evaluate the antibacterial activity of the fractions of *C. guianensis* and *A. monophylla* against nine clinical bacterial isolates namely *Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilius,* three

Gram positive bacteria and *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera and Proteus vulgaris six* Gram negative bacteria. The screening of these three fractions reveals that, fraction I, II and III and these fractions were subjected to disc diffusion assay against the bacterial pathogens the zones of inhibition obtained for the various micro-organisms showed remarkable activity against the micro-organisms tested. The increasing prevalence of antimicrobial drug-resistant microorganisms recovered from hospitalized patients is a major concern worldwide (Struelens, 1998) [11].

However, with so many antibacterial agents available for the treatment of systemic infections, treatment of bacterial infection is no problem at present, but the ability of microorganisms to acquire the capacity for multi-drug resistance can negate the therapeutic effect of an entire family of antibacterial agents. Therefore, the search for new, safe and effective antibacterial agents is necessary. Plants could be a good source for this purpose, and many plant extracts are reported to have anti-microbial activities (Naqvi *et al*., 1991; Sabahi *et al*., 1987; Almagboul *et al*., 1988) [12, 13, 14]. In the present study both the fractions of *C. guianensis* and *A. monophylla* were effective in all the tested bacteria. Among the fractions, marked antibacterial activity was displayed by leaf fractions II. Infectious diseases of microbial origin caused by *Staphylococcus aureus, Bacillus cereus, Shigellia spp* constitute the major causes of morbidity and or mortality in several countries (Kloos and Zein, 1993) [15]. Such microbial infection and pathophysiology in water and electrolyte transport could lead to diarrhoea. The fractions of *C. guianensis* and *A. monophylla* showed effective repressing activity against *Staphylococcus aureus* bacteria. Ramalakshmi *et al*., 2013 [16] conjointly showed antimicrobial property of methanolic extract *Couroupita guianensis* flowers. However, the therapeutic potentials of some of these botanicals have not been scientifically evaluated (Havagiray *et al*., 2004) [17]. It would be interesting therefore to search for plants with antimicrobial activities that could be used against infectious diseases.

Table 1: Antibacterial activity of fractions of *Couroupita guianensis* tested against the important selected human pathogenic bacteria

Fractions tested	Type	Pathogens tested	Concentrations tested			
			10 µl/ml	20 µl/ml	30µl/ml	*Control
			Zone of inhibition (mm)			
Fraction 1	Gram positive	*Staphylococcus aureus*	17	19	29	29
		Streptococcus epidermidis	10	12	17	27
		Bacillus subtilius	12	17	27	24
	Gram negative	*Escherichia coli*	19	24	28	27
		Klebsiella pneumoniae	10	15	20	22
		Pseudomonas aeruginosa	15	19	21	23
		Salmonella typhi	11	10	17	26
		Vibrio cholerae	9	15	19	24
		Proteus vulgaris	11	17	22	27
Fraction 2	Gram positive	*Staphylococcus aureus*	25	33	39	30
		Streptococcus epidermidis	17	19	23	28
		Bacillus subtilius	19	23	37	26
	Gram negative	*Escherichia coli*	19	21	35	28
		Klebsiella pneumoniae	15	19	25	22
		Pseudomonas aeruginosa	15	19	23	24
		Salmonella typhi	15	15	21	26
		Vibrio cholerae	13	17	19	24
		Proteus vulgaris	17	19	27	28
Fraction 3	Gram positive	*Staphylococcus aureus*	15	21	31	30
		Streptococcus epidermidis	11	17	21	29
		Bacillus subtilius	13	19	29	26
	Gram negative	*Escherichia coli*	15	17	27	28
		Klebsiella pneumoniae	13	15	21	23
		Pseudomonas aeruginosa	11	15	19	24
		Salmonella typhi	11	13	15	26
		Vibrio cholerae	9	10	13	24
		Proteus vulgaris	13	17	23	26

Values showing in the table are zone of inhibition obtained through disc diffusion method; Control = commercially available chemical drug: *Gentamycine (30µg/ml; for *E. coli, S. aureus,B. subtilius* and *P. aeruginosa* (Hailu Tadeg *et al*., 2005) [7], Ofloxacin (10µg/ml for *P. vulgaris, K. pneumoniae* and *S. typhi;* Karman *et al*., 2002) and Chloremphenicol (30µg/ml for *P. vulgaris, V. cholerae* and *S. epidermids*; Nancy *et al*., 2000) [8] were used as reference standards.

Table 2: Antibacterial activity of fractions of *Atalantia monophylla* tested against the important selected human pathogenic bacteria

Fractions tested	Type	Pathogens tested	Concentrations tested			
			10 µl/ml	20 µl/ml	30µl/ml	Control
			Zone of inhibition (mm)			
Fraction 1	Gram positive	*Staphylococcus aureus*	9	13	17	27
		Streptococcus epidermidis	7	9	10	25
		Bacillus subtilius	7	12	14	22
	Gram negative	*Escherichia coli*	12	15	18	26
		Klebsiella pneumoniae	11	15	19	21

		Pseudomonas aeruginosa	7	12	13	24
		Salmonella typhi	7	12	15	20
		Vibrio cholerae	12	15	17	25
		Proteus vulgaris	7	11	12	22
Fraction 2	Gram positive	*Staphylococcus aureus*	19	21	26	28
		Streptococcus epidermidis	14	15	18	25
		Bacillus subtilius	9	15	17	23
	Gram negative	*Escherichia coli*	17	21	28	26
		Klebsiella pneumoniae	15	21	26	21
		Pseudomonas aeruginosa	15	19	21	24
		Salmonella typhi	14	15	19	20
		Vibrio cholerae	13	17	20	25
		Proteus vulgaris	14	17	19	22
Fraction 3	Gram positive	*Staphylococcus aureus*	14	19	21	27
		Streptococcus epidermidis	11	15	19	25
		Bacillus subtilius	9	13	17	23
	Gram negative	*Escherichia coli*	14	17	21	28
		Klebsiella pneumoniae	10	14	19	21
		Pseudomonas aeruginosa	7	11	13	24
		Salmonella typhi	6	13	15	20
		Vibrio cholerae	13	17	19	25
		Proteus vulgaris	11	13	17	22

Values showing in the table are zone of inhibition obtained through disc diffusion method; Control = commercially available chemical drug: *Gentamycine (30µg/ml; for *E. coli, S. aureus,B. subtilius* and *P. aeruginosa* (Hailu Tadeg *et al*., 2005) [7], Ofloxacin (10µg/ml for *P.vulgaris, K. pneumoniae* and *S. typhi;* Karman *et al*., 2002) and Chlorempheniсol (30µg/ml for *P. vulgaris, V. cholerae* and *S. epidermids*; Nancy *et al*., 2000) [8] were used as reference standards.

Conclusion

Based on the studies, it may be concluded that *C. guianensis* and *A. monophylla possess* a wide range of antibacterial activities, in both crude and fractionated extracts. Also, new antimicrobial drugs can be developed for treating various diseases from the selected plant. Further, the individual active compounds can be isolated by chromatographic techniques and the fractions shall be evaluated separately for TLC, NMR to identify the compound functional group, nature and structure for converting as a new active drug.

Acknowledgement

Authors are very much thankful to Department of Science and Technology, SERB (Ref. No. SB-EMEQ-382/2014), Dew Delhi, India for financially supported to carried out this work.

References

1. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2011; 8:1-10.
2. Sumathi S, Anuradha R. Couroupita guianensis Aubl: An updated review of its phytochemistry and pharmacology. Asian Journal of Pharmacy and Pharmacology. 2017; 3(1):1-8.
3. Chung KT, Thomason WR, Wu-Yuan CD. Growth inhibition of selected food borne bacteria, particularly Listeria monocytogenes, by plant extracts. Journal of Applied Bacteriology. 1990; 69:498-503.
4. Bauer AW, Kirby WMM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standard single disc method. American journal of Clinical Pathology. 1966; 36:493-496.
5. Barry AL, Thornsberry C. Susceptibility tests. Disc diffusion test procedures. In: Lennette Balows, E.H., Hauster, W.J., Shadomy, H.J.(Eds.), Manual of Clinical Microbiology. American Society for Microbiology, Washington, DC, 1985, 978-987.
6. Lee E-W, Chen J, Huda MN, Kuroda T, Mizushima T, Tsuchiya T. Functional cloning and expression of emeA, and characterization of EmeA, amultidrug efflux pump from Enterococcus faecalis Biol. Pharm. Bull. 2003; 26:266Ð270.
7. Hailu Tadeg, Endris Mohammed, Kaleab Asres, Tsige Gebre-mariam. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders, Journal of Ethno Phamacology (In press), (On line search), 2005.
8. Nancy S, Mohammad Y, Abdallah S, Marie Elisabeth B, Mohammad R, Stephane B. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. FEMS Immunology & Medical Microbiology. 2000; 28(3-1):257-263.
9. Tringali C. Identification of bioactive metabolites from the bark of Pericopsis (Aformosia) laxiflora. Phytochemical Analysis. 2005; 6:289-291.
10. Mansouri S, Foroumadi A, Ghaneie T, Najar AG. Antibacterial activity of the crude extracts and fractionated constituents of Myrtuscommunis. Pharmaceutical Biology. 2001; 39:399-401.
11. Struelens JM. Tracking the epidemiology of antimicrobial drug resistance in hospitals: Time to deploy molecular typing. Journal of Medical Microbiology. 1998; 47:1035-1036.
12. Naqvi SAH, Khan MSV, Vohora SB. Anti-bacterial, anti-fungal and anthelimintic investigations on Indian medicinal plants. Filoterapia. 1991; 62:221-228.
13. Sabahi M, Mansouri S, Ramezanian M, Gholamhoseinian A. Screening of plants from the southeast of Iran for antimicrobial activity. International Journal of Crude Drug Research. 1987; 25:72-76.
14. Almagboul AZ, Bashir AK, Salih A, Karim M, Fairy A, Khalid SA. Antimicrobial activity of certain Sudanese plants used in folkloric medicine, screening for antibacterial activity (V). Fitoterapia. 1988; 59:57-62.
15. Kloos H, Zein AH. The ecology of health and

diseases. Washington DC: West View Press Inc, 1993.
16. Ramalakshmi C, Ranjitsingh AJA, Kalirajan K, Kalirajan A, Athinarayanan G, Mariselvam R. Elixir Applied Biology. 2013; 57:14055-14057.
17. Havagiray R, Ramesh C, Sadhna K. Study of antidiarrhoeal activity of *Calotropis gigantea* R.B.R. in experimental animals. Journal of Pharmacology and Pharmaceutical Sciences. 2004; 7:70-75.
18. Premalatha A, Karthi A. Phytochemical screening and antimicrobial activity of leaf of *Atalantia monophylla* (L.) Corr (rutaceae). International Journal of Pharmacy and Biological Sciences. 2017; 7(4):179-184.
19. Reddy KH, Sharma PVGK, Reddy OVS. A comparative invitro study on antifungal and antioxidant activities of Nervilia aragoana and Atlantia monophylla. Pharmaceutical Biology. 2010; 48(5):595-602.
20. Jones Ronald W, Stanley Engerman. Trade, Technology and Wages: A Tale of Two Countries, American Economic Review. 1996; 86(2):35-40.