Asian Journal of Research in Medical and Pharmaceutical Sciences

1(3): 1-9, 2017; Article no.AJRIMPS.35747

Phytochemical Screening and Antimicrobial Activities of Polar and Non-Polar Solvent Leaf Extracts of *Gongronema latifolium* (Asclepiadaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author KMAA designed the study and wrote the protocol. Author AMO handled all the laboratory bench work. Author SOB reviewed the experimental design and all drafts of the manuscript. Author FOI performed the statistical analyses and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2017/35747
Editor(s):
(1) BuLang Gao, Department of Medical Research, Shijiazhuang First Hospital, Hebei Medical University, China and Department of Radiology, Shanghai Jiaotong University Renji Hospital, China.
Reviewers:
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(4) Mustapha Umar, Nigerian Institute of Leather and Science Technology, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/21055

Original Research Article

Received 27th July 2017
Accepted 12th September 2017
Published 19th September 2017

ABSTRACT

Aim: This study was designed to assess the phytochemical constituents and antimicrobial activities of leaf extracts of *Gongronema latifolium* (Benth).
Methodology: The methods adopted were manual grinding of the air-dried leaves and maceration in polar and non-polar solvents (Ethanol and N-hexane) for 72 hrs. The resultant crude extracts were kept in dry, sterile airtight McCartney bottles and stored in the refrigerator. Thereafter, they were assayed for the presence of phytochemicals. Moreover, the plant extracts were screened for antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Aspergillus fumigatus*.
Results: The results of the phytochemical screening revealed the presence of saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids in the ethanol extracts while

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anthraquinones, steroidsand terpenoids were absent in the N-hexane extract of the plant. Moreover, the results of the antimicrobial activity assay of the plant extracts revealed a concentration dependent trend as higher activities was observed as the concentration gradient increased. *S. aureus* (20.33±0.01) and *E. coli* (19.67±0.00) showed the highest susceptibility to the plant extracts while *B. subtilis* (12.67±0.01) showed the least susceptibility against the plant extracts at 300 mg/ml. However, the plant extracts appeared not to have antifungal activity. The lowest minimum inhibitory concentration (MIC) was observed in ethanol extract against *E. coli* (6.25 mg/ml), while the highest MICwas recorded in N-hexane extract against *B. subtilis* and *S. typhi* (100 mg/ml). The ethanol extract of plant leaf was more active against the selected pathogens compared with N-hexane extract.

Conclusion: The outcome this investigation shows that the *G. latifolium* leaf extracts contain bioactive constituents such as saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids which may account for the antibacterial activities recorded.

Keywords: Gongronema latifolium; phytochemical; antimicrobial; pathogens.

1. INTRODUCTION

Traditional medicine also known as indigenous or (folk medicine), can be defined as the health practices, approaches, knowledge and beliefs incorporating plants, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being [1]. Traditional medicine has been used for thousands of years with great contributions made by practitioners to human health, particularly as primary health care providers at the community level. Countries in Africa, Asia, and Latin America use traditional medicine (TM) to help meet some of their primary healthcare needs. In Nigeria, for example, herbal medicine is the first line of treatment for 60% of children with high fever from malaria, while 85% of Nigerians use and consult traditional medicine for healthcare, social and psychological benefits [2]. Herbal remedies have a therapeutic effect and are acceptable interventions for diseases and symptoms. Interestingly, demand for medicinal plants is progressively rising in industrialized nations as it is in developing countries [3]. The world Health organization (WHO) has since urged developing countries to utilize the resources of traditional medicine for achieving the goals of Primary Healthcare. This has been due to the various advantages of traditional medicine namely: low cost, affordability, accessibility, acceptability and perhaps low toxicity.

Gongronema latifolium (Benth) commonly called ‘‘utazi’’ and ‘‘arokeke’’ in South Eastern and South Western parts of Nigeria respectively is a perennial edible plant with soft and pliable stem, belonging to the family of *Asclepiadaceae* [4]. It is widely used in the West African sub-region for a number of medicinal and nutritional purposes. It is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine [5]. A range of pharmacological tests have shown promising hypoglycaemic activities, and also interesting antibacterial, antioxidant, anti-inflammatory, hepatoprotective, antiplasmodial, anti-asthmatic, anti-sickling, anti-ulcer, analgesic and antipyretic activities of *G. latifolium* [6]. The leaves of *G. latifolium* are used as vegetables in preparation of soups to which they add a bitter-sweet flavor [7].

There are little reports on the antimicrobial efficacy of this multi-dimensional potent medicinal plant therefore, this study is aimed at assaying for the phytochemical and antimicrobial potency of the plant.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Extraction of the Extracts

Fresh *Gongronema latifolium* leaves were collected by plucking from the parent plants from Igbo-Oke forest in Ifon, Ondo state, Nigeria in November, 2016. The plant was then authenticated at the Herbarium section of the Department of Forest Resources Technology and a voucher specimen (XGL101) was deposited (in the same Department) Rufus Giwa polytechnic, Owo. The authenticated plant materials were washed and cleaned thoroughly with tap water and then air-dried under shade. The dried samples were then ground into coarse powder with the aid of a mechanical grinder and were stored in clean airtight containers, and kept in a cool, dry place until required for use.

One hundred gram (100 g) of the powdered sample was soaked in 300 ml of different solvents (ethanol and N-hexane) for 72 hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through Whatman No.1 filter paper into bijou bottles and then dried using rotary evaporator at a temperature of 50°C to yield crude extracts [8]. Different concentrations of the extracts were prepared by diluting 0.50 g, 1.00 g, 2.00 g and 3.00 g of the extracts in 100 ml of 0.01% Tween-20 to obtain concentrations of 50 mg/ml, 100 mg/ml, 200 mg/ml and 300 mg/ml respectively [9].

2.2 Test microorganisms

The test microorganisms used for this analysis include *Bacillus subtilis, Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Aspergillus fumigatus*. They were all collected from the Medical Microbiology Laboratory and Parasitology Department, Federal Medical Center, Owo, Ondo State, Nigeria in February, 2017. Organisms were sub-cultured and maintained on agar slants.

2.3 Qualitative Phytochemical Screening of *Gongronema latifolium*

The extracts of the plant leaves were subjected to qualitative phytochemical analysis for the presence of tannins, saponin, flavonoids, alkaloids and phenol using standard procedures as described by [10,11].

2.3.1 Test for tannins

A portion of1ml of extract was boiled in 20 ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observe the presence of green or a blue – black coloration which confirmed the presence of tannin.

2.3.2 Test for saponin

About 5 ml of the extract was boiled in 20 ml of distilled water in a water bath and filtered. A volume of10 ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirmed a positive presence of saponins.

2.3.3 Test for flavonoids

A volume of 3 ml portion of 1% Aluminum chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of dilute ammonia solution were added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicating a positive test for flavonoids.

2.3.4 Test for alkaloids

A 1 ml portion of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1 ml of the filtrate was treated with a few drops of either Mayer's reagent (Potassium mercuric iodide) solution gave a positive test for alkaloids.

2.3.5 Test for steroids

A 2 ml portion of acetic anhydride was added to 2 ml extract of each sample followed by careful addition of 2 ml H_2SO_4. The color changed from violet to blue or green indicating the presence of steroids.

2.3.6 Test for terpenoids (Salkowski test)

A volume of 5 ml of each extract was mixed with 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive result for the presence of terpenoids.

2.3.7 Test for anthraquinone

A 5 ml portion of extract was mixed with 10ml Benzene, filtered and 5 ml of 10% NH_3 solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammoniac (lower) phase indicated the presence of anthraquinones.

2.4 *In vitro* Antimicrobial Susceptibility Test

The extracts obtained from the test plants were screened against the test bacteria by agar well diffusion method [12]. A 25 ml aliquot of Mueller-Hinton agar (Lab Oratorios Britania, Argentina) and Sabouraud Dextrose agar (Oxoid, UK) was poured into different Petri plate. When the agar solidified, each test organism was inoculated on the surface the appropriate plates (1×10^6 *cfu*/ml) using a sterile glass spreader and allowed to sink

properly. Subsequently, the surface of the agar was punched with 6 mm diameter cork-borer into wells and a portion of 50 µl of each of the extract concentrations was filled into the wells. Control wells containing the same volume of Tween-20 served as negative control, while Chloramphenicol (50 µg) and fungisol (100 µg) were used as positive control for bacterial and fungal isolates respectively and the plates were incubated at 37°C for 24 h (bacteria) and 27°C for 72 hrs (fungi). Each experiment was carried out in triplicate and the diameter of the zones of inhibition was then measured in millimeters.

2.4.1 Determination of minimum inhibitory concentration (MIC) of *Gongronema latifolium* extracts

The minimum inhibitory concentration (MIC) of the plants extracts were determined by double dilution broth methods of Ghosh et al. [13]. Twofold serial dilutions of the extracts were prepared in Mueller-Hinton broth (for bacteria) and Sabouraud's Dextrose broth (forfungi) to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition (100 mg/ml to 1.56 mg/ml). All tubes with the controls were labeled accordingly. Each dilution was seeded with 1 ml of standardized inoculums (1.0×10^6 *cfu*/ml) and incubated at 37°C for 24 hr. A tube containing only seeded broth (i.e. without plant extract) was used as the positive control while the un-inoculated tube was used as negative control. The lowest concentration of each extract that showed a clear inhibition when compared with the controls was considered as the MIC.

2.5 Data Analysis

Data were presented as mean±standard error (SE). Significant difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version 17.0 software. The significance was determined at the level of $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents of *G. latifolium*

The phytochemical constituent screening of the *G. latifolium* leaf extracts revealed the presence of plant constituents such as saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids which varied according to the extracting solvents (Table 1). All the tested phytochemicals were detected in ethanol extract whereas anthraquinones, steroids and terpenoids were absent in N-hexane extract. The presence of various metabolites in the plant materials could justify its medical use [14]. Most of these compounds are also well known for their large spectrum of pharmacological properties, including antimicrobial (alkaloids and saponins) and antioxidant (tannins) activities [15,16]. This result is also in line with the report of Eleyinmi [17], who carried out the Chemical Composition and Antibacterial activity of methanolic extract of *G. latifolium* leaves and obtained similar results.

Table 1. Phytochemical constituent of *G. latifolium* leaf

Phytochemical	Ethanol	n-hexane
Saponins	+++	++
Flavonoids	++	++
Alkaloids	++	+
Anthraquinones	+	-
Tannins	+	+
Steroids	+	-
Terpenoids	+	-

Key: +++= present in abundance, ++= present moderately, += present in trace amount, -= not detected

3.2 Antimicrobial Activities of *G. latifolium*

The results of the antimicrobial activities of the plant extracts were concentration-dependent as higher activities was observed as the concentration gradient increased. The extracts exhibited different degrees of antimicrobial activity against tested organisms. Only *S. aureus* and *E. coli* were susceptible to the extracts at the lowest concentration used (50 mg/ml), all the bacteria were susceptible at 100, 200 and 300 mg/ml whereas none of the fungi was susceptible to the extracts at all the concentrations used. This is similar to the findings of Omodamiduro and Ekelemo [18] who reported that the ethanolic leaf extract show a significant dose dependent inhibition of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *E. coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

In all, ethanol extract of the plant showed higher antimicrobial activities than the N-hexane extracts. At the highest concentration used (300mg/ml), the ethanol extracts of the plant

showed comparable activities with the pure commercial antibiotic (chloramphenicol) used as positive control against *S. aureus* and *E. coli* compared with N-hexane extract which recorded a rather low activity against all the test organisms. This research work is in consonance with the findings of Morebise and Fafunso [19] who reported the antimicrobial activity of Utazi (*G. latifolium)* on *E. coli* and *S. aureus*. It might also be due to the differences in the concentration of the phytocompounds of various secondary metabolites present in the extracts as well as the extracting ability of the solvents. It therefore implies that polar solvent (ethanol) may be a better extraction solvent for the leaf of this plant than non-polar (N-hexane) solvent. This corroborates the observations of Abo and Ashidi [20].

The minimum inhibitory concentration (MIC) is the least concentration of the extracts that inhibit growth of organisms. It is an important diagnostic tool since helps in confirming resistance of microorganisms to antimicrobial agents. The lowest MIC was observed in found in ethanol extract against *E. coli* (6.25 mg/ml), while the highest was recorded in N-hexane extract against *B. subtilis* and *S. typhi* (100 mg/ml). This disagrees with the report of Nwinyi et al. [21] who reported zones of inhibition between 6 and 10 mm while minimum inhibitory concentrations (MIC) were 10.0 and 2.5 mg/ml respectively for the aqueous and ethanolic extracts of *G. latifolium* against *E. coli* and *S. aureus* respectively. This suggests that this plant may be useful in the management of intestinal pathogens especially the *Enterobacteriaceae* and to treat some related microbial infection.

Table 2. Minimum inhibitory concentration of extract of *G. latifolium* against test pathogens

Microorganism	Ethanol (mg/ml)	n-hexane (mg/ml)
Bacillus subtilis	75	100
Staphylococcus aureus	12.5	25
Salmonella typhi	75	100
Escherichia coli	6.25	25
Candida albicans	ND	ND
Aspergillus fumigatus.	ND	ND

Key: ND= not detected

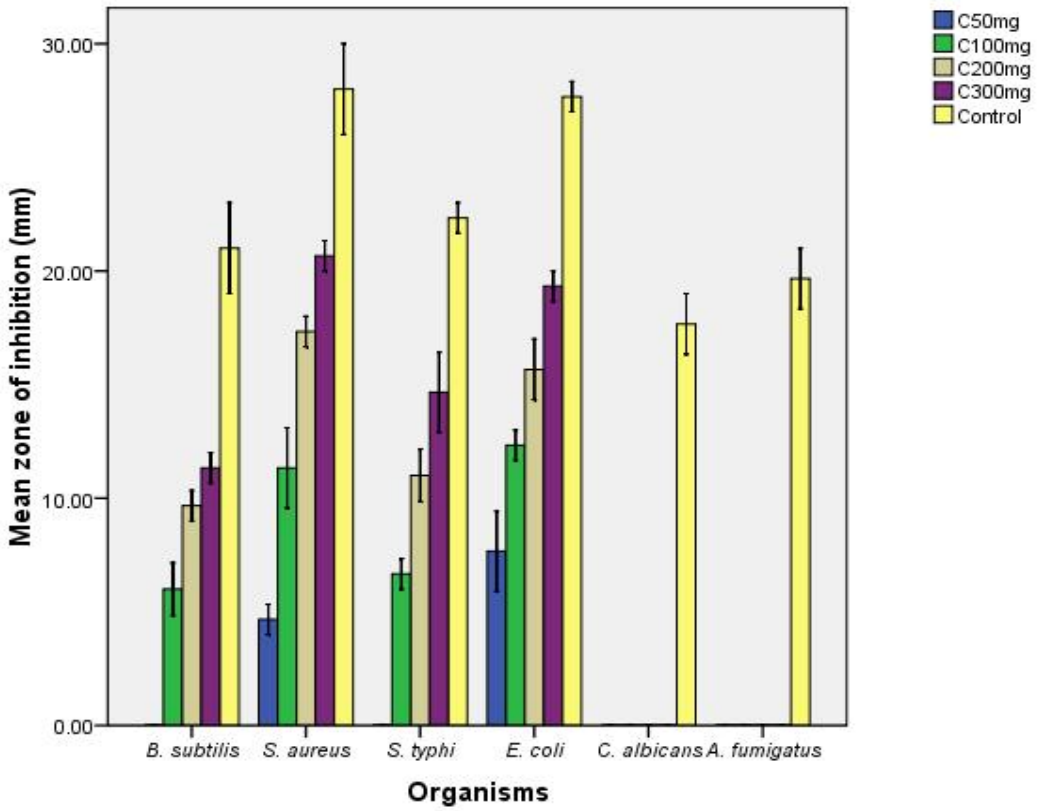

Fig. 1. Antimicrobial activity of ethanol extract of *G. latifolium* against test pathogens

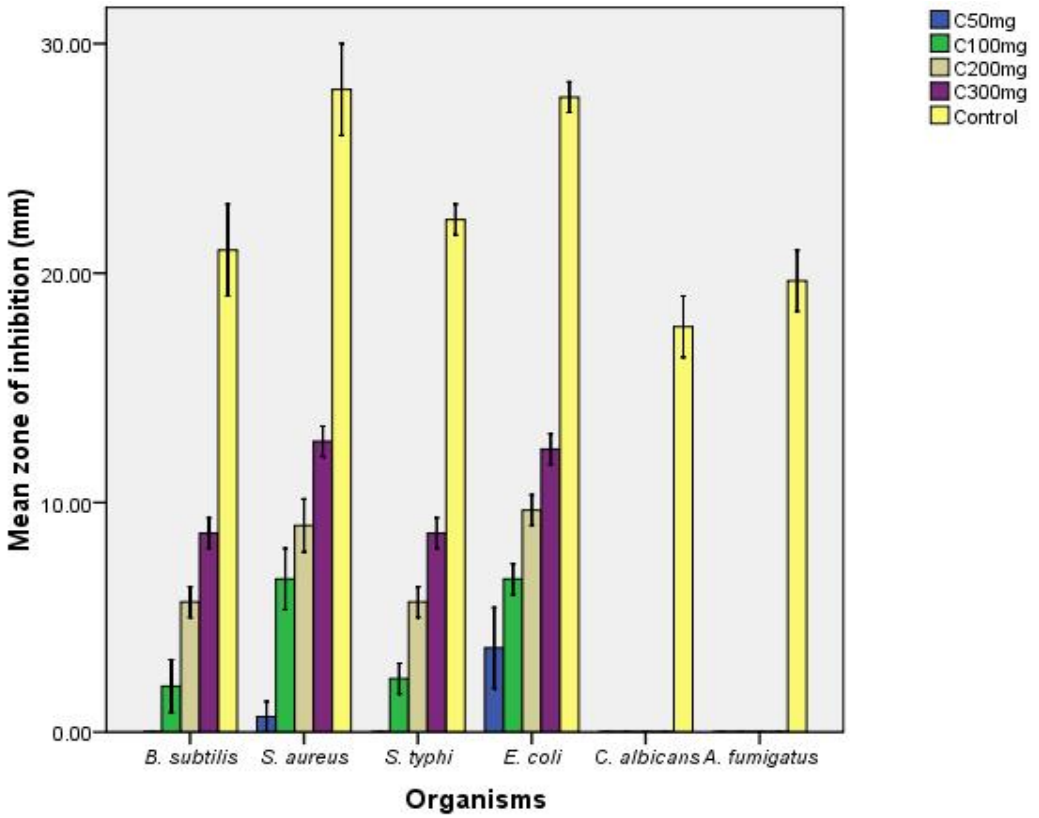

Fig. 2. Antimicrobial activity of n-hexane extract of *G. latifolium* against testpathogens

4. CONCLUSION

From the results obtained in this study, ethanol and N-hexane extracts of *G. latifolium* leaves contain bioactive phytochemicals like saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids. Moreover, the extracts possess antibacterial activity at higher concentrations against the test bacterial pathogens while it was notactive against any of the fungal species tested. Finally, polar solvent (ethanol) is a better extraction solvent for this plant than non-polar (N-hexane) solvent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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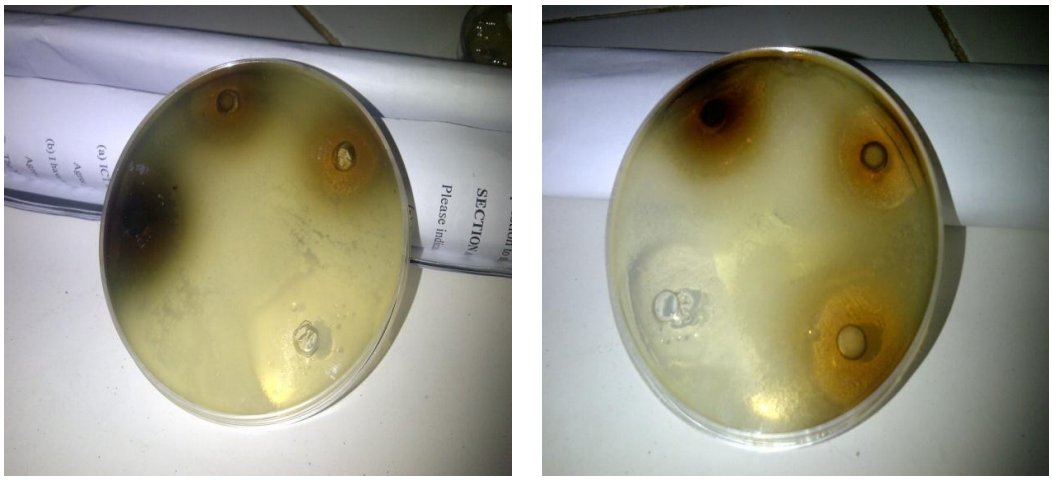
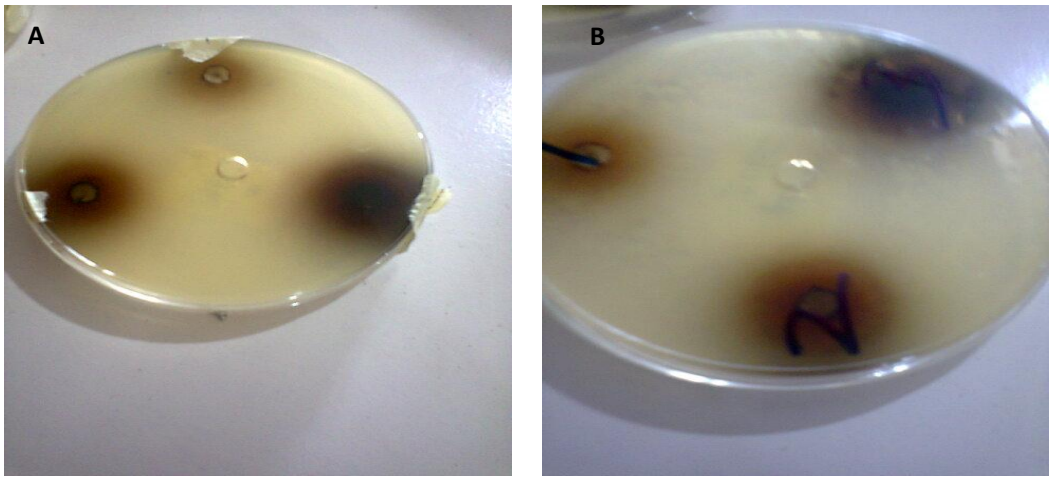
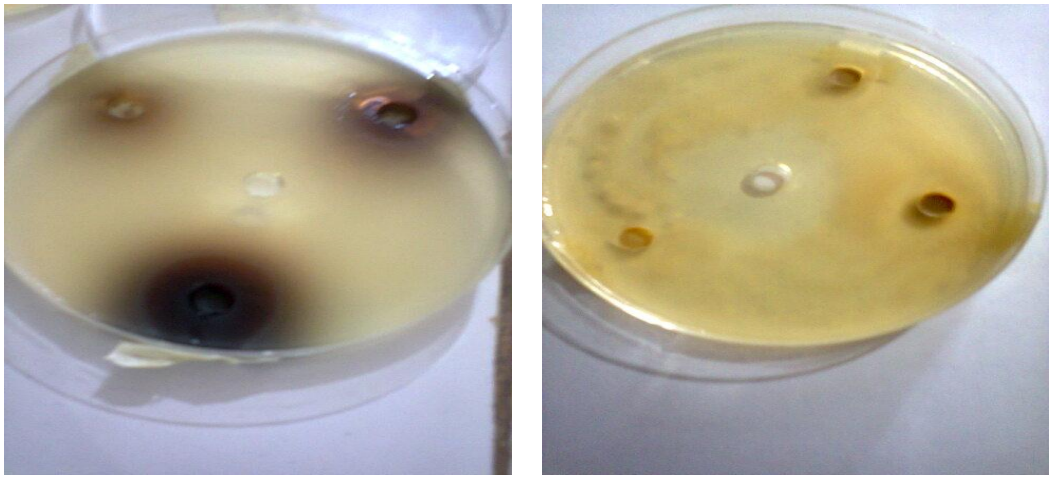
APPENDIX



Antibacterial activity of *G. latifolium* against *S. aureu* A= ethanol, B= n-hexane



Antibacterial activity of *G. latifolium* against *E. coli* A= ethanol, B= n-hexane



Antibacterial activity of *G. latifolium* against *B. subtilis*

Antifungal activity of *G. latifolium* against *A. fumigatus*

Antifungal activity of *G. latifolium* against *C. albicans*

Peer-review history:
The peer review history for this paper can be accessed here:
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