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COMPARATIVE STUDY OF PRELIMINARY ANTIMICROBIAL ACTIVITY OF THREE DIFFERENT PLANT EXTRACTS

Mohammad Nazmul Alam[1*], Md. Hasibur Rahman[1], Md. Jainul Abeden[1], Md. Faruk[1], Md. Shahrear Biozid[1], Sudipta Chowdhury[1], Md. Rafikul Islam[1], Mohammed Abu Sayeed[1]

[1]Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, 154/A, College Road, Chittagong-4203, Bangladesh.

***Corresponding author e-mail:** nazmul_pharmacy@yahoo.com

ABSTRACT

Disk diffusion method was performed to evaluate the ex-vivo comparative study of preliminary antimicrobial activity of methanolic extract of *Thunbergia grandiflora*, *Breynia retusa* and *Nymphaea capensis* leaves. Among the three plants, *T. grandiflora and N. capensis* showed more antibacterial activity than *B. retusa*. *T. grandiflora* showed its highest activity against a gram positive bacterium *Bacillus cereus* with the inhibition ring of 17 mm in diameter at 1000 µg/disc. In case of *B. retusa*, highest activity was found against the gram negative bacterium *Salmonella typhi* which is 16 mm at 1000 µg/disc. *N. capensis* exhibited its highest antibacterial activity against the gram negative bacterium *Escherichia coli* which is 19 mm at 1000 µg/disc.

KEYWORDS: *T. grandiflora*, *B. retusa*, *N. capensis*, antimicrobial activity, comparative study, disc diffusion method, Kanamycin.

INTRODUCTION

In this world microorganisms are the main reason for mortality and morbidity [1]. Antimicrobial agents are essential to treat these microorganisms. Plant derived antimicrobial agents are used as therapeutic tools to treat lots of pathogens. Chemical and synthetic products are costly and also cause various side effects as well as adverse effects. A great opportunity to overcome bacterial resistance is the combination of antibiotics with plant derived compounds and also discovering new antimicrobial agents from plants [2]. Our present investigation for the latest antimicrobial agents is targeted on the undiscovered medicinal plants which are used for thousands of years to treat local population [3]. In this study, *Thunbergia grandiflora*, *Breynia retusa* and *Nymphaea capensis* were tested against the target microbes, taking standard Kanamycin as positive control, to explore new plant derived antimicrobials. *T. grandiflora* Roxb. (Acanthaceae) is a large climbing or twining shrub, which is found widely all over the world such as India, China, Indo-China, Myanmar and many tropical countries of Africa [4]. It is also found throughout the Bangladesh, especially in forests of Gajipur, Chittagong, Chittagong Hill Tracts, Cox's Bazar, Tangail [5]. Generally, it is also known as black clock vine or blue trumpet vine, as well as many other common names in Bangladesh [4]. This plant is used for the treatment of blood dysentery, cataract, conjunctivitis, diabetes, gout, hydrocele, hysteria, malaria, marasmus, ophthalmia, post eclampsia, pre-eclampsia, rheumatism, spermatorrhoea, stomachache, stomach complaints, eye diseases (ophthalmia and conjunctivitis) and elephantiasis, eye diseases, stomach complaints and urinary bladder stone [6-9]. *B. retusa* is a shrub with spreading branches. It grows generally in Bangladesh [10] and other tropic Asian regions [11]. In Bangladesh, it is widely distributed in scrub forests of Sylhet and Chittagong Hill Tracts. The plant is used as astringent to the bowels and also useful in inflammations and diseases of the blood. The juice of the stem is used in conjunctivitis. Leaves are employed to hasten suppuration [10]. *N. capensis* is an aquatic flowering plant of the water lily

family Nymphaeaceae. Native to Africa, the plant is found growing abundantly in freshwater habitats in tropical regions of Africa, and as an introduced species in Australia, the state of Florida, and other tropical areas [12] such as Bangladesh, Mayanmar and India.

MATERIALS AND METHODS

Plant collection and identification: Leaves of the evergreen vine *T. grandiflora,* the deciduous shrub *B. retusa* and *N. capensis* were collected from different parts (Bhatiyari, Batali Hill and Anowara) of Chittagong region, Bangladesh in September of, 2013 and only leaf of these plants were used for this comparative study. The plants were identified by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong.

Preparation of extracts: Leaves of the plant materials were dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40-80mesh, 500 g) and soaked for 7 days with 2–3 days interval in 2.0 L of methanol at room temperature (23 ± 0.5°C). Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50°C using rotary evaporator (RE 200, Sterling, UK). The extracts (yield 4.4–5.6% W/W) were all placed in glass Petri dishes (90 X 15 mm, Pyrex, Germany). The extract was then preserved in a refrigerator till further use.

Chemicals and Reagents: Methanol and Kanamycin (30 µg/disc) were purchased from Merck (Germany) and Oxoid (England) respectively.

Ex-vivo Antimicrobial activity

Test organisms: Five pathogenic bacteria were selected for this experiment, three of which were Gram-negative and the remaining were Gram-positive. *Pseudomonas aeruginosa, Salmonella typhi*, *Escherichia coli* were gram-negative and *Staphylococcus aureus and Bacillus cereus were* gram-positive. These organisms of pure culture were collected from the Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

Preparation of the media: The instant nutrient broth was prepared from purified powdered agar, which were weighed and then reconstituted with distilled water in a conical flask according to specification. It was then heated in a water bath to dissolve the agar until a transparent solution was obtained. In this way nutrient broth was prepared.

Antimicrobial activity testing by disc diffusion method: Antimicrobial activity was performed by using disc diffusion method [13-14]. Circular discs of 5 mm in diameter were made from Whatman No. 1 filter paper (China) by using a punch machine. The filter discs were autoclaved at 180º C for 30 min and then each disc was soaked with 50 µl of each of the three different plant extracts at two different concentrations (500 µg and 1000 µg) by using micropipette. All of the discs were aseptically placed over the plates of agar media containing the agar media inoculated with test microorganisms. At last the plates were incubated at 37º for 24 hours. After 24 hours, diameters (in mm) of the inhibition rings were measured. Kanamycin (30 µg/disc) was used as standard and it was compared with the three methanolic plant extracts. Zones of inhibition with diameter less than 12 mm were considered as having low antimicrobial activity. Diameters between 12 and 16 mm were considered as moderately effective and diameters more than 16 mm were considered as highly active [15].

RESULTS AND DISCUSSION

The three different leaf extracts showed different zones of inhibition at two different concentrations (500, and 1000 µg/disc) against two gram-positive and three gram-negative bacteria. This experiment was performed by using disk diffusion method to evaluate the antimicrobial activity of these leaves. Among the three leaves, *T. grandiflora* and *N. capensis* showed more antibacterial activity than *B. retusa*. *T. grandiflora* showed its highest activity against a gram positive strain *Bacillus cereus* which presented a ring of 17 mm in diameter at 1000 µg/disc and no zone of inhibition was found against the gram negative *Escherichia coli*. In case of *B. retusa*, highest activity was found against gram negative *Salmonella typhi* with a ring of 16 mm in diameter at 1000 µg/disc and no zone of inhibition was found for the gram negative bacterium *Escherichia coli* as well as the gram positive bacterium *Staphylococcus aureus*. *N. capensis* exhibited its highest antibacterial activity against the gram negative *Escherichia coli* with a ring of 19 mm in diameter at 1000 µg/disc and no zone of inhibition was found for *Pseudomonas aeruginosa*. Kanamycin exhibited significant antimicrobial activity against all microorganisms. Zone of inhibition of Kanamycin was highest against *Salmonella typhi* and lowest against *Bacillus cereus*. The discovery of effective antibiotics has decreased the disastrous impact of

infectious diseases as well as enriched the quality of life. Nevertheless, the efficacy of many antibiotics is being endangered by the emergence of microbial resistance to existing chemotherapeutic agents because of their inappropriate use. The use of some antibiotics is associated with side effects, including allergy, immune suppression, and hyper-sensitivity. Lots of poor people are deprived from modern medicine because of high cost. For all these reasons, it is high time to identify new, safe, and cost-effective antimicrobial agents that would help to alleviate the problems of infectious diseases. Plant-derived natural products represent an attractive source of antimicrobial agents because they are natural and affordable, especially for rural societies [16-18]. This is only a preliminary study but these methanolic leaf extracts can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates.

CONCLUSION

Comparative study of methanolic extracts of *T. grandiflora, B. retusa and N. capensis* leaves exhibited moderate, minimal and promising antimicrobial activity respectively. From the above experiment, it could be stated that these methanolic plant extracts could be useful as therapeutic agents in preventing the antimicrobial diseases. Further studies are needed for their active principle to elucidate.

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Conflict of interest statement

Authors have none to declare.

Table 1. Antimicrobial activity of three different plant extracts by disc diffusion method

Serial No	Microorganisms	Gram positive/ Gram negative	Zone of Inhibition in mm						
			TG		BR		NC		Kanamycin (Standard)
			500 µg/disc	1000	500	1000	500	1000	30
1	*Pseudomonas aeruginosa* (ATCC 15442)	Gram negative	10	13	11	15	00	00	27
2	*Salmonella typhi* (AF 250878)	Gram negative	10	15	10	16	11	15	30
3	*Escherichia coli* (ATCC 25922)	Gram negative	00	00	00	00	12	19	28
4	*Staphylococcus aureus* (ATCC 259233)	Gram positive	09	13	00	00	12	14	29
5	*Bacillus cereus* (ATCC 10987)	Gram positive	12	17	09	13	09	13	26

*TG= *Thunberbia grandiflora*, BR= *Breynia retusa*, NC= *Nymphaea capensis*.

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