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Antimicrobial activities of *Bridelia retusa* (L.) Spreng. belonging to family Euphorbiaceae

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Abstract

The *in vitro* antimicrobial activity of the crude methanolic and ethanolic leaf extract of *Bridelia retusa* (L.) Spreng. was studied against four gram negative and two gram positive bacteria. The results showed that both the extracts showed antimicrobial activity. Maximum zone of inhibition was observed in the methanolic extract against *Escherichia coli* which was 29.5 ± 0.50. However, ethanolic extract showed least activity against *Staphylococcus aureus.* The basis of this plant extract in the traditional treatment of diarrhea in human is highlighted.

Key-Words: *Bridelia retusa*, Antimicrobial activity, Euphorbiaceae

Introduction

Herbal use in the treatment of animal and human diseases has long been recognized. Most plant extracts have been shown to possess anti-microbial agents active against micro organisms *in vitro*. These plants contain medicinal properties which make them effective to cure or prevent diseases (Wisdom and Shittu, 2010). It is anticipated that about 80% of the population use traditional medicine for their primary health care in developing countries (Mahmood *et al.*, 2011, Khan & Khan 2004 and Khan and Chagtai (1979).

There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have been developed due to arbitrary use of commercial antimicrobial drugs commonly used in the treatment of transmittable diseases. This situation has forced scientists to search new antimicrobial substances in various sources like medicinal plants (Jeyachandran *et al.*, 2010). Ekdania (*Bridelia retusa* L.) is a monoecious, deciduous plant belonging to family Euphorbiaceae that can grow to 8-10 m in height. Euphorbiaceae family in the plant kingdom is a complex hetero-geneous family consisting of about 322 genera and 8900 species in the world (Singh *et al.*, 2011). Little is known about the biological activity of most species of the genus *Bridelia.*

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The modern pharmacological studies of *B. retusa* (L.) Spreng. showed that it has a broad range of physiological activity and pharmacological effects, such as antibacterial, antiinflammatory, blood pressure, cholesterol, etc (Chen *et al.*, 1987). A number of infectious disease are caused by variety of bacteria some of them are pathogenic; others are opportunistic (Mahmood *et al.*, 2011). Some of the important bacterial pathogens are as follow: *Escherichia coli* causes' enteric infection, urinary tract infection and septicemia. *Staphylococcus aureus* causes pneumonia and cellulites. *Staphylococcus epidermidis* causes skin diseases in neonates. *Pseudo* sp. Causes urinary tract infection, nosocomial pneumonia, burns and wound infection. *Salmonella setubal* and *Bacillus subtilus* cause food poisoning. Antibacterial activities of various plant extracts were reported in the previous scientific reports using number of organic solvents like ethanol, methanol and petroleum ether. Our experiment was carried out with the organic solvents methanol and ethanol to find out the potentiality of *B. retusa*.

Material and Methods

Fresh leaves of *B. retusa*(L.) Spreng. were collected from the vicinity of Rewa (M.P.) India in February 2012 and were identified by The Herbarium of by the flora of Benthum and Hooker (1862-1883).

Preparation of plant extract

The dried leaves of *B. retusa* were powdered and sieved. The fine powder was stored in air tight containers and left in the refrigerator. Fifty grams powder were taken and soaked in 500 ml of solvent (methanol and ethanol). The poorly homogenized mixture was kept for two weeks at room temperature

(25°C). After three weeks maximum amount of solvent was separated from the mixture. Filtrate was filtered twice; first using ordinary filter paper and then Whatman #41 filter paper. The extracts were completely evaporated by rotary evaporator. About 2 g of *B. retusa* was obtained. Agar well diffusion method was used.

Microorganisms

Six bacterial strains were used; Two were gram positive, which were *S. aureus* (ATCC6538), *B. subtilis* (ATCC6633), and the four were gram negative, which were *P. aeruginosa* (ATCC6643) *E. coli* (ATCC15224), *K. pneumoniae* (MTCC618) and *S. typhimurium* (ATCC13048). The strains were obtained from the Department of Environmental Biology, A.P.S. University, Rewa (M.P.). These entire microorganisms were maintained on nutrient agar medium at 4°C.

Preparation of inocula

Colony of bacteria from 24 h old culture was taken and mixed in a nutrient broth prepared by dissolving 0.13 g of nutrient broth per 10 ml of distilled water and was placed in incubator for 24 h. After 24 h selected bacterial stain was mixed with autoclaved saline solution, made by dissolving 0.9 g NaCl/100 ml of distilled water and turbidity was corrected until a McFarland 0.5 $BaSO_4$ turbidity standard (10-6 colony forming unit per ml). Then this inoculum was used for seeding the nutrient agar plates.

Preparation of the seeded agar plates

Nutrient agar medium was prepared by suspending nutrient agar (Merck) in 1 L distilled water; pH was adjusted at 7.0 and was autoclaved. It was allowed to cool up to 45°C. Petri plates were prepared by pouring 75 ml of seeded nutrient agar and allowed to solidify. The plates were placed in incubator for 24 h. After 24 h, an inoculating loop or swab was dipped in broth and then streaked on agar plates in different directions. Ten wells per plates were made with sterile cork borer (8 mm).

Pouring, incubation and measurements of zone of inhibition

After 24 h by using micropipette, 100 µl of test solution was poured in respective well. The solution of antibiotics was made by dissolving 2mg of penicillin per ml of DMSO. Eight concentrations of extract were poured in eight wells; DMSO was used as negative control and antibiotic solution for positive control. Before putting all these extracts, respective wells were sealed by putting a drop of agar in order to avoid any mixing of solutions. These plates were incubated at 37°C. After 24 and 48 h of incubation, the diameter of clear zones, showing no bacterial growth, around each well was measured. All the measurements were taken in mm. Antibacterial activity of all dilutions of extracts was determined against six bacterial strains. All the materials used in these experiments were subsequently inactivated and autoclaved. All the assays were done in triplicate and the results were given in mean ± S.E.

Results and Discussion

Results of present investigation were presented in Tables 1 and 2. It was found that methanolic extract of *B. retusa* showed significant antimicrobial activity against *E. coli* with inhibition zone 29.5 ± 0.50 mm at the concentration of 15 mg/ml, whilst the extract showed inhibition against *S. aureus, P. aeruginosa* and *S. typh* with inhibition zones 24 ± 1.00, 21 ± 1.00 and 23 ± 1.00 mm, respectively at concentration 15 mg/ml. When the concentration of the extracts was decreased, slight decrease in inhibition zone was observed. Ethanolic extract of *B. retusa* also exhibit strong inhibitory effect against *E. coli* and the zone of inhibition observed was 18 ± 1.00 to 26 ± 1.00 mm at the concentration of 1 to 15 mg/ml. However, all other bacterial strains (*K. pneumoniae, B. subtilis, S. typh, S. aureus* and *P. aeruginosa*) showed moderate zones of inhibition which lies between the 14 ± 1.00 to 24 ± 1.00 mm at different concentrations.

The results of the present study revealed that the methanolic and ethanolic extracts of leaves of *B. retusa* possess appreciable potentiality of inhibiting the growth of all the strains of bacteria at different concentrations however, methanolic extract showed better results as compared to ethanolic extract. The results of the present study revealed that the methanolic extract possess appreciable potentiality of inhibiting the growth of all the strains of bacteria, it is also supported by Vlachos *et al.* (1996) and Chandrasekaran and Venkatesalu (2004) which concluded that methanol was the most effective solvent for the extraction of antimicrobial compounds from the selected seaweeds. The anti microbial activity of ethanolic leaf extract of *B. retusa* on different bacterial strains may be related to the antibacterial effect of this plant. The findings in this study that the ethanolic leaf extract showed inhibitory effect at different concentrations showed the potentials of the plant in the treatment of bacterial infection due to bacterial organisms. This is in accordance with the previous findings of Chen (2008). Usually, plant material is used as a crude extract and such treatments do not aim at using the pure isolate of the extract. The work demonstrated *in vitro* the antimicrobial activity of the crude extract of *B. retusa* leaves against the organisms used in this study. However, it displayed a basis for the use of the extract by practitioners in the treatment of

diarrhea in human which could be caused by different bacterial spp. It is suggested that further research should be carried out to investigate the bioactive constituent of this plant. This is necessary to investigate the toxicity level of the extract resultant from over dosage or from any of the phytochemical component present in the plant material.

Conclusion

Our experimental results provide further information regarding the organic solvent used for the extraction of plants. It was highlighted in our study that the methanolic and ethanolic extracts of *B. retusa* showed remarkable antibacterial effect against *S. typhi, E. coli* and *S. aureus.* Hence, it may be recommended that this plant could be used in the treatment of human diseases caused by the previously mentioned organisms. As *B. retusa* is an important medicinal plant and very little work has been done on this aspect, these findings will be new in research field.

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References

1. Benthum, G. and Hooker, J.D. (1862-1883). *Genera Plantarum* Vols. 1-3 London.
2. Chandrasekaran M, Venkatesalu V (2004). Antibacterial and antifungal activity of *Syzygium jambulanum* seeds, **91:** 105-108.
3. Chen G (2008). Antibacterial Activity of Fractions of Ethanol Extracts from Root Bark of *Bridelia retusa* against *Escherichia coli* from Swine. J. Anhui Agric. Sci., 2; 1234-1242.
4. Chen JS, Zheng (1987). Poisonous plant in China. Beijing, Science Press, p. 261.
5. Jeyachandran R, Baskran X, Cindrella L (2010). *In vitro* Antibacterial activity of three Indian medicinal plants. Int. J. Biotechnol., 1(1): 103-106.
6. Khan, A.A. and Shabina Khan 2004. Ethnobotanical significance of wild poisonous plants of Amarkantak, District Shahdol, Madhya Pradesh, India. *Ad. plant Sci.* **17(II)** 435-438.
7. Khan, S.S. and Chagtai, S.A. 1979. Euphorbias of Bhopal and their medicinal utility. *Chikitsa* 1(1): 75-76.
8. Mahmood A, Mahmood A, Shaheen H, Qureshi RA, Sangi Y, Gilani SA (2011). Ethno medicinal survey of plants from district Bhimber Azad Jammu and Kashmir, Pakistan. J. Med. Plants Res., 5(11): 2348- 2360.
9. Mahmood A, Mahmood A, Tabassum A (2011). Ethnomedicinal survey of plants from District Sialkot, Pakistan. J. Appl. Pharm., 02(03): 212- 220.
10. Singh B, Dutt N, Kumar D, Singh S, Mahajan (2011). Taxonomy, Ethnobotany and Antimicrobial Activity of Croton bonplandianum, *Euphorbia hirta* and *Phyllanthus fraternus*. J. Adv. Dev. Res., 2(1): 21-29.
11. Valchos V, Critchely AT, Van HA (1996). Establishment of a protocol for testing antimicrobial activity in southern African macro algae. Microbios. 88: 115-123.
12. Wisdom SGO, Shittu GA (2010). *In vitro* antimicrobial and phytochemical activities of *Acacia nilotica* leaf extract. J. Med. Plants Res., 4(12): 1232-1234.

Table 1: Antimicrobial activity of methanolic extract of *Bridelia retusa*

Conc.	Zone of inhibition					
(mg/ml)	***E. coli***	***B. sub***	***P. aeru***	***S. aur***	***K. pneu***	***S. typh***
15	29±0.50	17±1.00	18.5±3.50	24±1.00	17±1.00	21±1.00
12.5	27±0.50	16±0.50	21±1.50	19±0.50	16±0.50	19±1.00
10	26±1.00	16±1.00	21±2.00	20±1.00	13±0.50	17±1.00
7.5	26±1.00	16±1.50	20±1.00	19±1.00	15±1.00	15.5±1.50
5	24±0.50	15±1.00	16±1.00	18±0.50	13.5±1.50	-
3	25±1.00	-	16±1.00	18±0.50	11.5±1.50	-
2	20±0.50	-	-	19±1.00	11±1.00	-
1	19±0.50	-	-	16±1.00	-	-
A	35	27	28	33	30	28
DMSO	-	-	-	-	-	-

Key: *E. coli, Escherichia coli., B. sub, Bacillus subtilis, P. aeru, Pseudomonas aeruginosa, S. aur, Staphylococcus aureus, K. pneu, Klebsilla pneumonia. S. typh, Salmonella typhimurium,* A, Penicillin, DMSO, Dimethyl sulfoxide, _ , No activity, ±, represent the value of standard error.

Table 2: Antimicrobial activity of ethanolic extract of *Bridelia retusa*

Conc.	Zone of inhibition					
(mg.ml)	***E. coli***	***B. sub***	***P. aeru***	***S. aur***	***K. pneu***	***S. typh***
15	26±1.00	23.5±1.50	21±1.00	21±1.00	24±1.00	23±1.00
12.5	24±0.50	22±0.50	21.5±1.50	19.5±1.50	23.5±0.50	19±1.00
10	24±1.00	21±1.00	19±1.00	17.5±1.50	21.5±0.50	19±1.00
7.5	21±0.50	21±0.50	19±1.00	-	22±1.00	17±1.00
5	20±0.50	20±0.50	18±1.00	-	20±1.00	17±1.00
3	21.5±1.50	19.5±1.50	16±1.00	-	19±1.00	16±1.00
2	19±0.50	18±0.50	17±1.00	-	16±1.00	14±1.00
1	18±1.00	17.5±1.00	15±0.50	-	15±1.00	14±1.00
A	35	27	28	33	30	28
DMSO	-	-	-	-	-	-

Key: *E. coli, Escherichia coli, B. sub, Bacillus subtilis, P. aeru, Pseudomonas aeruginosa, S. aur,Staphylococcus aureus, K. pneu, Klebsilla pneumonia, S. typh, Salmonella typhimurium,* A., Penicillin, DMSO, Dimethyl sulfoxide, _, No activity, ±, represent the value of standard error.