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ROYAL CENTRE FOR DISEASE CONTROL
Department of Public Health | Ministry of Health | Royal Government of Bhutan

Brief Report

Antimicrobial activity of Artemisia collected in Serbithang, Thimphu

Tshering Dorji[1] and Vishal Chhetri[1]

[1]Royal Centre for Disease Control, Department of Public Health, Ministry of Health, Serbithang, Thimphu, Bhutan

Introduction:

Artimisia vulgaris is a species with a great importance in the history of medicine and was called "mother of herbs" in the middle age (1). It is a common herbaceous plant that exhibits high morphological and phytochemical variability depending on the location where it occurs. The different applications of this plant species have been possible due to its rich chemical composition, which especially includes essential oils, flavonoids, sesquiterpene lactones, phenolic acids, coumarins, and other groups of metabolites. Artemisinin was found to be a good antibacterial, antifungal, antileishmanial, and antitumor agent (2). The antibacterial properties of artemisinin had been tested on a wide range of bacteria, such as *Escherichia coli* (3), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Mycobacterium intracellularae* (4). Recently, this species has been taken under consideration to be active toward the virus SARS-CoV-2 and disease COVID-19 (5, 6).

Artemisia plant is very common in Bhutan. The extraction is prepared using the whole leaf, the leaf is pressed and the juice is extracted and applied externally on the skins either as an insect repellent or to treat skin diseases (7). There is also anecdotal that this plants extract can cure bone diseases and therefore used during hot stone bath. However, the lack of scientific evidence

to support this believes leave a gap between and knowledge and use. Thus, this experiment was preformed to assess the in-vitro antimicrobial property of Artemisia extract found locally.

Material and methods

Collection of plant material for extraction

The leaves of *Artemisia vulgaris* plant were collected from the surroundings of RCDC (Royal Center for Disease control). It was shredded into pieces and dried at 37 degrees Celsius for 120 hrs. Dried leaves were meshed into small particles and weigh 50gm of sample in two separate beakers. In one beaker 150ml of Water was added and, in another beaker, 150ml of Methanol was added. It was allowed to stand at rotatory incubator at 25degree Celsius/ 100RPM for 72hrs. Water and Methanol was dried using hot plate at 40 degrees Celsius for 120 hrs. The final extract was aseptically collected and stored at 2-4°C until use.

To test the antimicrobial property the filter paper disc size (6mm) was autoclaved and soaked into Artemisia extract for 24 hrs. The ABST (antibiotic susceptibility test) test was performed as mentioned by Chhetri., et.al (2022) (8) .

Microorganism

The Following strains of Bacteria were used in the study was obtained from Enteric and Invasive Disease Laboratory (EIDL), RCDC:

1. ATCC25922- *Escherichia Coli* (Gram Negative)
2. ATCC 25923 - *Staphylococcus aureus* (Gram positive)
3. ATCC 28523 - *Pseudomonas aeruginosa* (Gram Negative)

Antibacterial Activity

Mueller Hinton agar media was prepared and plates were swabbed for 24hrs cultures of respective bacteria grown in nutrient broth. The extracts were tested for antimicrobial activity using disc diffusion method by Van. et al (9). Sterile discs of approx. 8mm diameter were prepared from each extract of methanol mixture and water mixture and 3 discs from each extract were impregnated on the media and then plates were incubated at 37 degrees Celsius for 24 hrs.

After the incubation period, each plate was observed for zone of inhibition and measured using transparent scale.

Results and Discussion

Figure 1: Antimicrobial property of Artemisia extract

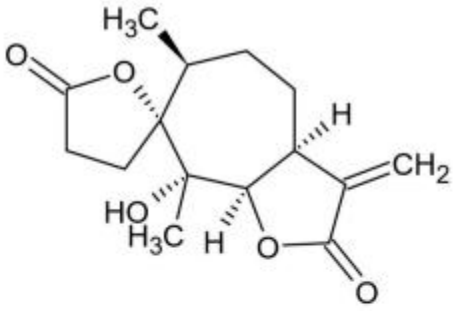
Table 1: Antibacterial screening (Zone of inhibition, diameter in mm)

Bacterial strain	Methanol extract	Water extract
Escherichia Coli (ATCC-25922)	6±0.1mm	9 ±0.1mm
*Staphylococcus aureus (*ATCC-25923)	14±0.3mm	12±0.6mm
Pseudomonas aeruginosa (ATCC-27853)	19±0.3mm	17±0.6mm

The results of the antibacterial activity of Artemisia were demonstrated but with different size of zone of inhibition (ZoI) to different micro-organisms. The highest size of ZoI was presented with the methanol extract as compared to the water extract. Methanol extract had highest ZoI in *Pseudomonas aurigenosa (*19±0.3mm) and lowest ZoI to *E.coli* strain (6±0.1mm).

Amongst the three different types of organism, *P. aurigenosa* presented with highest size of ZoI 19±0.3mm (methanol extract) and 17±0.6mm (water extract), whereas *E.coli* had the lowest size of ZoI in both water and methanol extract (9 ±0.1mm and 6±0.1mm).

Variety of Artemisia plant species is common in different parts of the country. Plant extract had been used for long since due to its antimicrobial property, the other active compound present in plant extract are phenolic compounds and antioxidants. This phyto-constituent present in the extracts may be responsible for the antimicrobial activity. In the current experiment the methanol extract showed higher range of ZoI in different organism, though no significant difference between Gram negative and Gram positive bacteria. The chance to find antimicrobial activity was more apparent in (methanol) alcohol extract than in water (10).



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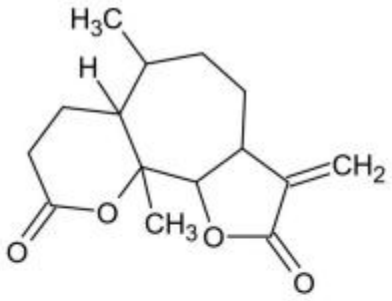

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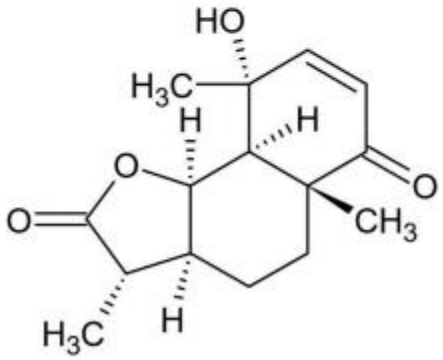

Figure 2. Chemical structure of sesquiterpenoid characteristic of *A. vulgarigas*

Conclusion:

The ability of *Artemisia vulgaris* extract to inhibit microbial growth suggested that it possess antimicrobial activity against different microorganism. The extracts from Methanol are more effective for *Pseudomonas aeruginosa* and *staphylococcus aureus* than *Escherichia coli.* However, the extract from water is more effective against *Escherichia coli* than from Methanol extract. The biological activities of *Artemisia vulgaris* raw material proven so far have raised hopes for a revival of the interest of contemporary medical world.

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