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Evaluation of Antimicrobial Activity, Taxonomic characteristics and Ethnobotanical Uses of *Osyris wightiana* Wall. ex Wight

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ABSTRACT: *Osyris wightiana* Wall. ex Wight, commonly known as Jangli Chai, is an evergreen shrub or small tree belonging to Santalaceae family. The majorities of plants are partially parasitic on other plants and are widely distributed in the tropical and temperate region. The plants are traditionally used to treat a variety of health issues, including gynaecological disorders and treating broken bones, among other things. The leaves are used to make a wild herbal tea.

The present study has been conducted to access the antimicrobial activity, taxonomic characteristics and ethnobotanical uses of *Osyris wightiana* Wall. ex Wight. The leaf extract of *Osyris wightiana* were screened for antibacterial activity against two standard strains of bacteria i.e. *Pseudomonas aeruginosa* (1522) gram negative; *Streptococcus mutans* (497) gram positive. For the positive control, ampicillin was taken and DMSO was taken as negative control. The present investigation revealed that no antibacterial activity was exhibited by methanolic extracts of leaves of *Osyris wightiana*. But in accordance with earlier investigations, the references are available regarding the antimicrobial activity of *O. wightiana*.

Keywords: Osyris wightiana Wall. ex Wight, traditional uses, antibacterial activity, etc.

INTRODUCTION

In the recent years, the use of herbal medicines has been extensively expanded all over the world. More than 80% of world population directly or indirectly depends on the traditional herbal medicines for their primary health care (Gewali and Awale 2008).

The medicinal values of the plants are known due to the presence of some active chemical constituents (Egwaikhide and Gimba 2007). These bio-active compounds are responsible for anti-microbial effect of plant extract in-vitro, for e.g. flavonoids, alkaloids, glycosides, saponins, tannins, terpenoids, carbohydrates and sterols etc. (Hena et al., 2010; Ghosh et al., 2007). Thus, the utilization of plant extract and phytochemical, both with known anti-microbial properties may lead to play a significant role for the discovery of new drugs in therapeutical treatments. Nowadays, antimicrobials derived from the plants have been receiving the increasing attention because synthetic anti-biotics have shown ineffectiveness against several human pathogenic organisms due to increasing drug resistance (Akgul and Saglikoglu 2005). Therefore, a number of studies have been conducted for screening of plants in different countries to find out novel potent compounds for anti-microbial therapy (Jack and Okorosaye-Orubite 2008). Ethno-botanical knowledge of traditional health care has greatly attracted the interest of pharmaceutical

companies into research and developmental programs in the pursuit of novel drugs (Ogunnusi and Dosumu 2008).

As stated by World Health Organization traditional medicine can be defined as knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies to treat diagnose or prevent illness. Herbal drugs are unprocessed parts of plant while herbs include crude plant material such as flowers, leaves, fruits, seed, stem, wood, roots or other parts which may entire, fragmented or powdered (Birhan et al., 2017). According to the WHO more than 65 % of the world population have incorporated medicinal plants for treating diseases. The active compounds from these medicinal plants are antimicrobial agents that have the ability to fight bacteria and fungi. Bacterial infections are widespread and cause sickness. These bacterial pathogens continue to be the threat to human health and welfare as a result of new or resistant pathogens (Chand et al., 2017).

Natural products certainly have the potential to be used as source of antimicrobial drug discovery. There are around over 1340 plants with definite antimicrobial activities and also over 300,000 antimicrobial compounds have been isolated from plants (Ngwoke *et al.*, 2011). The plants are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, and polyphenols, which are generally superior in their antimicrobial activities (Cowan, 1999). The common metabolites that have a potential to have antimicrobial activities are alkaloids, fatty acids, phenolic components and terpenoids (Vandeputte, 2012).

Alkaloids are among the largest group of secondary metabolite being extremely divers in terms of structure and biosynthesis pathway, including more than 20,000 different molecules distributed throughout approximately 20% of known vascular plants. Alkaloids are low molecular weight nitrogen containing compounds and, due to the presence of heterocyclic rings containing nitrogen atom, are typically alkaline. Alkaloids are known by their numerous pharmacological effects on vertebrate. The metabolite can be divided into different classes according to their precursor encompassing more than 20 different classes e.g pyrrolidine alkaloid, tropane alkaloid, piperidine alkaloids, pyridine alkaloids, quinolizide alkaloid, and indol alkaloids (Matsuura and Fett-Neto 2015).

More than 1000 natural acetylic metabolites have been isolated from a wide variety of plant, fungal species and marine algae and invertebrates many of them display important biological activities, namely antitumor, antibacterial, antimicrobial, antifungal, and other medicinal properties (Kilimnik et al., 2016).

Phenolic compounds are the most widely distributed secondary metabolites. It is uncommon in bacteria fungi and algae. Higher plants synthesized several thousand known different phenolic compounds leaves of vascular plants contain esters, amides and glycosides of hydroxycinnamic acid, glycolated flavonoids; especially flavonols, proanthocyanidins and their relatives (Caretto et al., 2015).

Terpenoids are substances which give plants and flowers their fragrance. They occur widely in the leaves and fruity of higher plants, conifers, citrus and eucalyptus. The term terpenes are used to denote compounds containing an integral number of C5 units and chemically all terpenoids are derived from basic branched C5 unit (isoprene or 2-methyl-1,3-butadine). According to the number of such C5 unit present in the molecule, terpenoids are classified in to hemi-, mono-, sesqui-, di-, sester-, tri-, and tetraterpenoids (carotenoids) having 1,2,3,4,5,6 and 8 isoprenoids C5 residue respectively. A vast number of terpenoids have high potential on anti-inflammatory effect (Las Heras et al., 2003).

Phytochemicals employed by plants to protect them against pathogenic insects, bacteria, fungi or protozoa have found applications in human medicine (Nascimento et al., 2000). Plant can also exert either bacteriostatic or bactericidal activity. The variation of activity results is because of antimicrobial concentration of plant constituents present in plant organs due to one geographic location to another depending on age of the plant, nutrient concentration of the soil, extraction method, harvesting times, and antimicrobial analysis technique (Doughari, 2012).

The antimicrobial effect also depends on the physicochemical properties of antimicrobial agents (acid dissociation constant, solubility and hydrophobicity/lipophilicity ratio), the environmental Malik et al.. Biological Forum – An International Journal 15(3): 880-885(2023)

factors (PH, water activity, temperature) and microbiological factors like species genus and strain of the target microorganism (Juneja et al., 2012). The well-known mechanism of action of antimicrobial agents are interference with cell wall, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of intermediary metabolic pathways and disruption of the cytoplasm membrane (Tenover, 2006).

O. wightiana Wall. ex Wight, commonly known as Jangli Chai, is an evergreen shrub or small tree belonging to Santalaceae family. It is an important ethno-medicinal plant as well. Its synonyms are O. arborea Wall., O. arborea f. puberula Hook., O. divaricata Pilger, O. nepalensis Griff. In India, plant is distributed in Himachal Pradesh, Uttarakhand, Arunachal Pradesh, Nagaland, Manipur, Orissa, Madhya Pradesh, Andhra Pradesh, Goa, Kerala, Tamil Nadu, Sikkim etc. (Kumari et al., 2018).

O. wightiana is used in folk medicines and traditional Chinese medicines. Its leaves stem and roots are used for the healing of fractures (Bhattarai, 1992). Leaves are also reported for their emetic properties and antiviral activity (Chandel et al., 1996). Bark of the plant is used to make tea, which cures constipation and other stomach disorders. Besides these medicinal properties O. wightiana plants also possess antimicrobial and antioxidant properties, reported in various studies (Gairel et al., 2011 ; Preethi et al., 2010), but earlier studies didn't report antimicrobial and antioxidant properties of leaves of O. wightiana. Therefore, present study is focused on the exploration of antimicrobial activity of methanol leaf extracts of O. wightiana.

MATERIALS AND METHODS

A. Plant Material

The required plant part (leaves) was collected randomly from the Tallital area, 29°20' and 29°30'N latitude and 79°23' and 79°42' E longitude, in Nainital district of Kumaun Himalaya for the antiomicrobial analysis of O.wightiana. The plant material was collected in the month of June 2022. The plant material used for antimicrobial activity were thoroughly washed off under running tap water to get rid of all the debris and soil and shade dried at room temperature for two weeks. The air-dried plant material was finely grinded into powdered and packed in self seal air tight polythene bags for further use (Upadhyay, 2011).

B. Preparation of plant extracts

The fresh leaves of O. wightiana were washed under running tap water. The plant material was allowed to dry naturally *i.e.*, under shade drying at room temperature and powdered. After completion of drying process, material was ground in a grinder and the powder was kept in an appropriately labelled plastic bottle. Five grams leaf extracts are dissolved in 80% methanol. Incubated for 8 days at 37°C in shaker incubator, thereafter the extracts were filtered and the filter was evaporated to obtain the final residue. The final residue was dissolved in 25% DMSO (Dimethyl sulfoxide) after that the extract was kept in a

refrigerator at 0° C to determine the antimicrobial activity (Upadhyay, 2011).

C. Antimicrobial Assay

Antimicrobial assay of extracts of *O. wightiana* was performed by disc diffusion method in Nutrient Agar plates. The test organisms were inoculated in Nutrient broth and incubated overnight at 37° C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×108 CFU/ml. NA plates were plated with 100µl of microbial culture broth. Plant extracts of 100 mg/ml concentration were prepared in Dimethyl Sulfoxide (DMSO). Discs of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm). Each well was filled with different concentrations of extracts were added to the disc.

Positive control (ampicillin (50µg) and negative/solvent control (25% DMSO an diol propanol), respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C.

D. Antimicrobial test

Cultures of bacterial strains *Pseudomonas aeruginosa* 1522 and *Streptococcus mutans* 497 were revived and grown in LB medium. Nutrient agar plates were prepared. 100 μ l of each stain were plated in nutrient agar plates in duplicates for each stain (for each extract) and 6mm discs were loaded with different concentration of the extracts the solvent, when the solvent was dried from the disc, it was loaded on agar plates and incubated at 37°C for overnight. For positive control different concentration of ampicillin was used.

RESULTS AND DISCUSSION

The genus *Osyris* comprises of the evergreen shrubs or trees belonging to the sandal wood family, *Santalaceae*. The majorities of plants are partially parasitic on other plants and are widely distributed in the tropical and temperate region. It includes more than 34 species (Xiang *et al.*, 2003). The fruit is a nut or a drupe and brightly colored. The fruit is relatively small (up to 1 cm), usually with rudimentary perianth at apex or with only rudimentary disk, exocarp fleshy, endocarp crustaceous.

Inflorescences are axillary, male ones cymose; bisexual and female ones often 1-flowered; bracts and bracteoles on male inflorescences caduceus. Flowers are unisexual (when plants dioecious) or are bisexual. The bisexual flowers have perianth tube mostly connate to ovary, lobes 3 (or 4), triangular or ovate, with a tuft of hair adaxially. Short stamens are 3 (or 4) on the base of lobes. Anthers are ovoid; cells are distinct, parallel, and dehiscence longitudinal. Disks are nearly flat and sinuate at margin. Ovary is inferior, 1-loculed, and 2-4 ovules. Styles are needle shaped. Stigmas are 3 (or 4) parted. The male flower has stamens slightly longer than the bisexual flower and ovary is rudimentary. Female flowers have prominent bracteoles and perianth tube shorter. Seeds are globose.

The branches of *Osyris* are usually 3-ridged or -angled. Leaves are alternate, \pm sessile, elliptic-lanceolate or elliptic-ovate. Leaves are usually \pm leathery, pinnately veined and lateral veins inconspicuous (Shu, 2003).

A. Taxonomical Characteristics

*Osyriswightiana*Wallich ex Wight, Icon. Pl. Indiae Or 5: t. 1853. 1852; Santapau, Rec. Bot. Surv. India 16: 269. 1953; Babu, Herb. Fl. D. Dun 452.1977; Naithani, Fl. Chamoli 2: 562.1985. O. arborea Wallich ex DC., Prodr. 14: 633.1857; Hook. F. in Fl. Brit. India 5:232.1886

Distribution: Submontane and montane Himalaya from Himachal Pradesh to Bhutan, Central and S. India; Sri Lanka & Myanmar.

Altitude: upto 2400 meters

Leaves: Alternate, subsessile or short petioled, ellipticoblanceolate.

Inflorescence: Bisexual, small, polygamous, pale green; Male flowers are 8-10 in axillary umbellate cymes whereas Female or 2-sexual flowers solitary, axillary and pendulous.

Flowering: March – May Fruiting: August – November Scientific classification Kingdom: Plantae Phylum: Tracheophyta Class: Magnoliopsida Subclass: Rosidae Order: Santalales Family: Santalaceae Genus: Osyris

B. Ethnobotanical Uses

The genus *Osyris* is widely distributed in the tropical and temperate region of southern Europe, northern Africa, and southwest Asia. Leaves, roots, barks, fruits, and woods of the shrub are used for different purposes by ethnic groups of different region of Asia, Africa, and Europe.

Leaves. The fresh leaves of O. compressa are used to tan the leather with light brown color, while the bark was used to tan the leather with dark brown color. A decoction of fresh leaves is used to tan cotton to make them more durable, in the days before nylon (Orwa et al., 2009). The Tamangs and Newars of Kavre, central part of Nepal, use leaves of O. wightiana as a substitute of tea. The leaves of plant are dried and its infusion in hot water is consumed as tea. O. wightiana is locally known as 'Nundhiki' in Kavre district of Nepal. The tea made from the leaves of O. wightiana stimulates the flow of breast milk and also acts as the labor-inducing agent (Osujih, 1993). Two kilograms of the leaves are crushed and boiled with constant stirring in 10 1 of water. When the amount is reduced to half the volume, it is stressed and the filtrate is evaporated again until a viscous sticky mass is obtained, called 'Nundhikikokhoto'. It is cooled and preserved. The drug (Nundhikikokhoto) is mixed with double the amount of cow's butter or Indian rapeseed oil (Brassica napus) and used to massage the sprained parts twice a day for relief (Raboanatahiry et al., 2021).

Immature leaves and fruits have emetic properties and contain tannins. It also contains cis-4-hydroxy-L-proline and exhibited antiviral activity. Leaf guises can be used in the veterinary purposes (Aryal and Pelz 2008). The leaves of *O. lanceolata* (Hocst and Stendel) are boiled and served orally in the traditional

management of ear, nose, and throat (ENT) diseases (Njoroge and Bussmann 2006).

Roots- Roots and barks of *O. lanceolata* are used for the tea and as a tonic in the soup. The root decoction is used to treat diarrhea in Kenya. The decoction of bark and heartwood is used to treat sexually transmitted diseases and anemia in Tanzania. Extracts from the plant can cure certain diseases, including the killer Hepatitis B. In central parts of Nepal, the root paste and bark of *O. wightiana*, is used to plaster around the fractured bone after adjusting it properly. About 100 g of bark is boiled in 3 l of water for an hour to obtain a gelatinous mass and is applied around the dislocated bone. The plaster in then wrapped in a cloth. The whole structure is kept together with the help of thin bamboosplints (Bhattarai, 1990).

The root bark is boiled in water for about 10 min, cooled, and stressed; this liquid, about 10 tea spoons three times a day, is given to women after childbirth to stop bleeding and to boost energy, which helps in contraction of uterine muscles (Shrestha and Joshi 1993). The roots are used in case of tingling (Jhamgarne in Nepali) or neurological problems (Acharya and Rokaya 2005). The roots of O. quadripartite are used for the treatment of cancer in Tanzania (Graham *et al.*, 2000). *O. alba* is used for making brooms in Italy. The root fibers are also used to make baskets (Orwa *et al.*, 2009).

Bark- The barks of the stem and roots of *O. compressa* are used in the manufacture of aromatic oils that are used in making expensive perfumes, quality lotions, rare soaps, and sweet-smelling candles. The wood yields between 4 and 10% oil when distilled. The boiled concoction of the bark, together with other herbs, is

reputed to be useful in improving blood circulation, digestive, and respiratory and nervous systems. The boiled product is given to women after giving birth to boost their appetite. The oils and paste derived from the bark are used to treat skin diseases such as infectious sores, ulcers, acne, and rashes.

The tree is also known to be a disinfectant and a sedative. It is effective against the killer hepatitis B. The bark when boiled produces a dark color solution, which is used to flavor the tea (Orwa *et al.*, 2009). The bark is boiled in water, stressed, and boiled again to form a gelatinous mass that is applied in the eyes to relieve inflammation. About 1 teaspoon of this preparation, three times a day, is given to persons suffering from blood dysentery. The juice of bark, and in some places a paste of fruit, is applied to forehead to relieve headache. The juice of bark, about 4 teaspoons three times a day, is given in cases of indigestion (Manandhar, 2002). An infusion of the bark is emetic. Decoction of the bark is taken to treat diarrhea (Wantanabe *et al.*, 2005).

Fruits. The fruits of *O. compressa* are edible and are an important food of early inhabitants of the South African cape. The powdered fresh/dry fruit of *O. quadripartite*, mixed with water, is given orally for 3 days and applied topically on the infected body part of livestock in Wonago Woreda of Ethiopia (Fisseha *et al.*, 2009).

Wood. The heart wood scent is used in sacred ceremonies and to purify holy places. Incense sticks from the wood are burned in temples and houses. The wood is heavy and fine grained, suitable for curving ornaments, and small utensils like pestles (Orwa *et al.*, 2009). The heart wood is faintly fragrant and reported to be used for adulterating sandalwood.



C. Antibacterial activity of the extract

The leaf extract of *Osyris wightiana* were screened for antibacterial activity against two standard strains of bacteria *i.e. Pseudomonas aeruginosa* (1522) gram negative; *Streptococcus mutans* (497) gram positive. For the positive control, ampicillin was taken and DMSO was taken as negative control. The present investigation revealed that no antibacterial activity was exhibited by methanolic extracts of leaves of *Osyris wightiana*. But in accordance with earlier investigations, the references are available regarding the antimicrobial activity of *O. wightiana*.

control, ampicillin was taken and
s negative control.Kumari *et al.* (2018) tested the antimicrobial activity of
methanol and chloroform leaf extracts of *O. wightiana*
in vitro against two bacterial pathogens (*E. coli* and *S.*Biological Forum – An International Journal15(3): 880-885(2023)883

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typhimurium) and the results of antibacterial study showed that the plant is effective against *E. coli* and *S. typhimurium*.

The pathogenic strains of *E. coli* cause diarrhea, abdominal pain and fever while *S. typhimurium* cause gastroenteritis in humans. There are many reports on

antioxidant, antimicrobial and anticancer activity of plant and the antimicrobial activity of plant are associated with the presence of alkaloids (Govindarajan *et al.*, 2003). There are reports that flavonoids possess multiple biological property including antimicrobial, cytotoxicity and anti-inflammatory (Bors *et al.*, 1990).



Photo Plate : Antimicrobial activity of methanolic extract of *Osyris wightiana* (leaf against bacterial pathogens, a: *Pseudomonas aeruginosa*; b: *Streptococcus mutans*)

CONCLUSIONS

The present study has been conducted to access the antimicrobial activity, taxonomic characteristics and ethnobotanical uses of Osyris wightiana Wall. ex Wight. The leaf extract of Osyris wightiana were screened for antibacterial activity against two standard strains of bacteria i.e. Pseudomonas aeruginosa (1522) gram negative; Streptococcus mutans (497) gram positive. For the positive control, ampicillin was taken and DMSO was taken as negative control. The present investigation revealed that no antibacterial activity was exhibited by methanolic extracts of leaves of Osyris wightiana. But in accordance with earlier investigations, the references are available regarding the antimicrobial activity of O. wightiana.

FUTURE SCOPE

Osyris wightiana could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

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Conflict of Interest. None.

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