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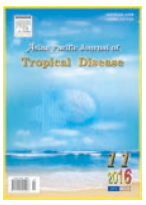

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Pharmacological aptitude and profiling of active constituent from *Otostegia limbata*-Comprehensive review

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ABSTRACT

The plants provide medicinally valuable substances since ancient time and are the cheapest, oldest, as well as imperative source of pharmacologically active chemical compounds used for the treatment of several diseases. Medicinally, *Otostegia limbata (O. limbata)* is very imperative species of genus *Otostegia* which are extensively utilized by traditional practitioners against several ailments and its chemical constituents possess antispasmodic, antiulcer, antidepressant, sedative, anxiolytic, anti-inflammatory for eyes inflammation, antibacterial, antioxidant, haemagglutination activity, cholinesterase inhibition for Alzheimer's treatment, antitussive potential, anti-aphids and larvicidal potential aptitude. The pharmacological impact of *O. limbata* and their diverse biological activities along with its active constituent are complied in this review. This species provide rich variety of biologically active secondary metabolites. Quite large number of active compounds isolated from *O. limbata* is the evidence to have considerable potential making them competent candidate for critically required novel drugs.

1. Introduction

The consumption of herbal medicines has always been a crucial part of human culture, because some plants possess significant therapeutic potential, which can be utilized to treat human and other animal ailments. Natural products are source of various imperative life saving drugs which used in the armamentarium of many modern medicines. Though, among almost 400 000 plant species, no more than 6% have been investigated for biological interest and among them just 15% have been evaluated phytochemically. This reveals the requirement for planned activity and directs phytopharmacological assessment of herbal medicines[1].

2. Taxonomic hierarchy

Genus *Otostegia* belongs to the family Lamiaceae which is one of the biggest and most distinctive families of flowering plant species with 220 genera and almost 4 000 plant species throughout the world[2]. *Otostegia* consists of about 33 plant species and in Pakistan, just three species have been discovered that are *Otostegia limbata (O. limbata)*, *Otostegia persica* and *Otostegia aucheri*. *Rydingia limbata* (Benth.) Scheen & V.A.Albert and *Ballota limbata* Benth are synonyms of *O. limbata*. The common and vernacular names of plant species are "Spin aghzai", "Spin azghay", "Bui", "Phut kandu", "Chiti booti" and 'Chitti jharri"[3].

3. Morphological description

O. limbata is tiny bush, greatly branched, slender shape and 2 feet tall spiny shrub. Plant has yellowish or brownish stem which is quadrangular on new shoots with short eglandular hairs. Main branch is erect, divided, spiny, woody and white or may be gray colored bark[4]. Leaves of plant are oblong oblanceolate, crenate or dentate, clustered, attenuate at base, obtuse at apex, small with short petiole and spiny bracts. *O. limbata* bears pale yellow (Figure 1), clusters and long axillary flowers bloom (flowering period) in April-May[3].

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Figure 1. *O. limbata.*

4. Geographical distribution

O. limbata is extensively dispersed in the lower hills of west side of Punjab and northwest of Frontier Province in Pakistan[3]. In Pakistan, *O. limbata* is commonly spread in hilly region of Punjab and northwest of Frontier Province. *O. limbata* is also found in diverse part of Northern Pakistan together with Siran Valley of Mansehra, Malam Jaba, along Lahore to Islamabad (M2) motorway[5-7]. *O. limbata* also distributed in Northern West of Pakistan in hilly part near Abottabad[8].

5. Mineral composition

Several trace elements present including different macronutrients and micronutrients which were presented in Table 1. Plant leaves contain noticeably high amounts of calcium and potassium whereas the quantity of sodium is relatively insignificant. This may be the reason to make this plant species valuable in controlling hypertension and decoction of the leaves is utilized to overcome potassium and calcium deficiency. Leaves of the plant also contain zinc and copper are valuable to maintain ocular function and retinal problems as well as the antioxidant enzymes functioning. Plant contains significant amount of iron which is integral part of hemoglobin[9].

Table 1
Trace elements in *O. limbata.*

Trace elements			
Macronutrients elements	mg/100 g	Micronutrients elements	mg/100 g
Na	45.000	Fe	52.700
K	393.400	Mn	8.000
Ca	1 972.000	Zn	4.600
Mg	37.200	Cu	0.847
-	-	Ni	0.672
-	-	Cd	0.059

6. Ethnopharmacology

Medicinally, *O. limbata* is a very imperative species of genus *Otostegia* which is extensively utilized by traditional practitioners against several ailments, and its chemical constituents possess antispasmodic, antiulcer, antidepressant, sedative and anxiolytic aptitude[10]. *O. limbata* is consumed for the treatment of children's gum problems, for remedial purpose in case of ophthalmia in man and also used for the cure of wounds in beast and man[11]. Locally fresh leaves of *O. limbata* are crushed and then grounded, after that water is mixed to make the extract which is also used for the cure of eye infections[8] and also utilized for treatment of the bleeding gum in children[12].

7. Pharmacological significance

The plants provide medicinally valuable substances since ancient time and are the cheapest, oldest, as well as imperative source of pharmacologically active chemical compounds used for the treatment of several diseases[13]. Local herbs based remedies are being remarkably consumed by quite a large number of people inhabiting rural area for several purposes. However, serious extensive efforts are needed for scientific justification related to consumption of medicinally crucial plants for extenuating the sufferings which are caused by quite large considerable number of sicknesses[14]. Genus *Otostegia* is the imperative source of natural products for pharmacological active. The pharmacological impact of *O. limbata* and their diverse biological activities along with its active constituent are complied in this review. This species provides rich variety of biologically active secondary metabolites. The crude form as well as different fractions or extracts belong to *O. limbata* are good anti-inflammatory and antimicrobial agents. A quite large number of active compounds isolated from *O. limbata* is the evidence to have considerable antimicrobial potential making them competent candidate for critically required novel antibiotics[3].

7.1. Anti-inflammatory potential (against eye inflammation)

The *O. limbata* is therapeutically functional for a variety of diseases including eye infections and inflammations. The aqueous leaves extract of *O. limbata* is utilized for the cure of eye inflammation and the eye infection in Abbottabad (Pakistan). The efficacy of this extract against eye ailments may be because of, inter alia, the present of minerals or due to antimicrobial potential. Mineral composition of this plant is evident for the reason of its folk utilization for treatment of eye diseases. The aqueous leaves extract of *O. limbata* contains almost 4.600 mg/100 g of Zinc. This significant trace element was found to be part of around 70 different enzymes. The function of zinc in eye healthiness is imperative. It plays integral role for the maintenance of normal eye function. Zinc is the abundant trace element in eyes and its intensity is 15 times more than in retina as compared to the circulation[15]. The eye manifestations of Zn deficiency comprise oscillatory potentials, electroretinograms and altered vision. However, in case of acute

deficiency, ultrastructural alterations are noticed in the retina along with retinal pigment epithelium. Progression of a few degenerative retinal disorders may be altered by Zn supplementation[16]. Zn is also one of imperative antioxidant elements and therefore, can provide protection against damage caused by free radicals[9]. Zn ions also have astringent effect and slightly antiseptic potential, so they can be utilized for the treatment of eye ailments in forms like zinc sulfate. One of the renowned ocular drops named 'Oculosan' have 0.2 mg/mL of zinc sulfate[14].

7.2. Antibacterial activity

The ethanolic extract of *O. limbata* possesses significant antibacterial potential against several Gram-positive bacterias including *Staphylococcus saprophyticus*, *Staphylococcus epidermidis* (two strains), vancomycin-resistant *Enterococcus faecalis* (*E. faecalis*) and methicillin-resistant *Staphylococcus aureus* (*S. aureus*). There is no considerable activity against Gram-negative bacterial strains including *Klebsiella pneumoniae* (2 strains), *Providencia rettgeri* (2 strains), *Enterobacter cloacae*, *Escherichia coli* (3 strains), *Kluyvera* spp., *Morganella morganii* and *Klebsiella terrigena* (4 strains). The reason for dissimilarity in sensitivity of Gram-positive bacteria and Gram-negative strains may be ascribed to the morphological disparity between the phospholipids membrane bearing structural components making cell wall impermeable to lipophilic solutes as porins comprise a selective barrier to hydro-philic solutes[17]. Gram-positive strains possess an external peptidoglycan coating, an effective permeable film[18]. *O. limbata* has constituent of broad spectrum antibacterial agent specifically against Gram-positive bacteria[19]. Antibiotic drug resistance is raising a dilemma all over the world particularly in developing countries owing to the unsystematic use of antibiotics. Physicians are not having broad choice of antibiotics for treatment of infections triggered by potentially particular pathogenic and several multidrug resistant strains such as methicillin-resistant *S. aureus* which are responsible for many human diseases such as endocarditis, septic arthritis, staphylococcal scalded skin syndrome and vancomycin-resistant *E. faecalis* cause of endocarditis, urinary tract infections, pelvic infections, intra-abdominal infections, skin and soft tissues infection, wound infections, bacteremia and catheter-related infections[19]. Likewise, *Staphylococcus saprophyticus* is the reason of urinary tract infection especially in women and *Staphylococcus epidermidis* is the leading pathogen of nosocomial infections. Both of these bacterial strains are resistant to broad spectrum antibiotics owing to mutation adaptability[20]. So, there is urgent necessity to find some alternative sources of efficient antibacterial substances which could be developed into potent antibiotics. *O. limbata* is a excellent candidate to discover new alternative source particularly against the fatal pathogens methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecalis*[9].

7.3. Antioxidant activity

Significant antioxidant aptitude in the Lamiaceae plants provides evidence about the information that expands the knowledge of achievable mechanism that motivates their traditional utilization. The free radical scavenging potential of methanolic extract of *O. limbata* reported in terms of radical scavenging or hydrogen donating ability through the stable radical 1,1-diphenyl-2-picrylhydrazyl. 1,1-Diphenyl-2-picrylhydrazyl values illustrate the strength of *O. limbata* to neutralize the free radicals which can trigger cancer. The absorbance of the *O. limbata* extract with 0.22629 and percentage scavenging activity as 68.96% reveals considerable antioxidant potentials. The chemical compounds that are responsible to inhibit oxidation process and carrying healthy attributes are an indication for providing cardiovascular health and found abundant in *O. limbata*[21].

7.4. Haemagglutination activity

O. limbata possesses effective haemagglutination potential against multiple blood groups by adding samples (1 mL) to 2% suspension of red blood cells (1 mL) and the mixture needs to be incubated at 25 ℃ in a water-bath. Rough granules in bottom and underside of test tube point toward agglutination of erythrocytes affirm positive activity whereas smooth button like formation shows sedimentation of erythrocytes and confirms negative activity of plant. Granular deposition determines the agglutination and can be organized into different following categories: a) trace or very weak; b) weak; c) moderate; d) strong[22]. *O. limbata* possesses haemagglutination activity of extracts and several fractions (5 mg/mL) in all blood-groups because of erythrocytes agglutination. However, concentrations lower than this ranges from 2.5000–0.3125 mg/mL showed negative activity in all blood groups. This potential activity in extracts as well as in different fractions indicates the presence of lectins which are responsible factors for haemagglutination[23]. Lectins are important useful agents for analyzing histochemistry[24], studying cell differentiation and for the isolation and characterization of glycopeptide and glycoconjugates[25]. Now a days, phytolectins have attained much attention due to their effectiveness in investigating cellular membranes[26]. This is also effective against several types of cancers. Phyto origin based lectins can be monitored and characterized to achieve valuable therapeutically sound effects[27].

7.5. Cholinesterase inhibition for Alzheimer's treatment

Acetyl-cholin-esterase (acetyl-cholinesterase 3.1.1.7) is an important key factor of neuromuscular junctions and cholinergic-brain-synapses. The main biological function of this enzyme is termination of the impulse transmission through rapid hydrolysis

of one of the cationic neurotransmitters called acetylcholine[28]. Cholinergic hypothesis reveals that memory impairments with this senile dementia issue in patients is because of selective and also irreversible insufficiency in the cholinergic functions in the brain[29]. There are three important tricyclic *cis-clerodane* type diterpenoids, namely, limbatolide A, B and C isolated from roots of *O. limbata* together with two renowned compounds, beta-sitosterol and oleanic acid. All above mentioned compounds exhibit inhibitory activity against butyryl-cholin-esterase and acetyl-cholin esterase, and vary in a concentration-dependent behavior. Exploitation of acetyl-cholin-esterase inhibitors act as rationale for the symptomatic therapy of Alzheimer's disease in its initial stages[30]. Function of butyryl-cholin-esterase is still elusive in normal ageing process and brain diseases. Butyryl-cholin-esterase is significantly higher in amount in case of Alzheimer's plaques as compared to plaques of common normal age associated with non-demented brains[31].

7.6. Antitussive effects

Airway inflammatory conditions such as asthma, cough variant asthma and eosinophilic bronchitis trigger numerous mechanical events in the wall of airways including smooth muscle contraction, vasodilatation and edema, mucus secretion, and decrease in lung compliance by release of different inflammatory mediators and these variations in the airways-wall boost coughing. *O. limbata* has diterpenoids which possess inhibitory activity against enzyme lipoxygenase[32]. These enzymes play a critical function in the biosynthesis of a number of chemical mediators including hepoxilins, leukotrienes, lipoxins and hydroxyeicosatetraenoic acid in mammalian cells along with an imperative function in airways inflammation and bronchial asthma[33]. *O. limbata* contains flavonoids which have significant antiallergic properties, and also possesses some derivatives such as disodium cromoglycate which is chromone renown for clinical utilization in the therapy of asthma and cough[34]. So the presence of flavonoids partially describes its antitussive potential[35] and this effect could be characterized as peripheral acting on efferent or afferent limb of cough reflex (spasmolytic and antiallergic). However, the likelihood of central exploitation by suppressing cough motor pattern-generator might not be excluded, keeping in mind that these responsible agents have the potential to provoke central nervous system activity[36].

7.6.1. Antitussive potential in mice

O. limbata has antitussive capability against cough in mice induced by different chemicals. The antitussive potential of this species correlates with several pharmacological characteristics which could justify its extensive utilization in various respiratory situations in folk medicines. Mild dosage of *O. limbata* extracts (400 mg/kg) significantly reduce frequency of cough provoked through SO_2 gas. Marked reduction in the number of cough efforts occurs before and after dosage from 48.1 to 33.5 after half an hour and significant decrease in 25.5 after 1 h. Administration of high dosage of extracts causes considerable inhibition in cough reflex as compared to dextromethorphan (10 mg/kg) and codeine (10 mg/kg). In numerous patients, no apparent cause related with persistent cough occurs, so it is essential to repress irritable cough by antitussive mediator[37]. Recently, antitussive was widely used including centrally acting narcotic based antitussive. They have apparent antitussive action at amount lower than those needed for relief in pain. Conversely, these antitussives agents cause a very high degree of unwanted effects, such as inhibition of ciliary drowsiness activity, constipation, decreased secretion in the bronchioles, nausea, physical dependence and depression of respiratory center[38]. These entire shortcomings usher the development of novel antitussive agents particularly non-narcotic, which possibly will prevent the pathological cough[36].

7.7. Anti-aphids and larvicidal potential

In Pakistan fruit pest like *Drosophila melanogaster* (*D. melanogaster*) infected many pulpy fruits, predominantly guava and banana. They feed on pulps of fruits consequently making the fruits worthless. In summer, their population is so large which cause a terrible deterioration of fruits[39]. In Pakistan, annually fruit flies grounds a loss of about 7 million rupees to growers[40]. Consequently, the control of these flies and many other pests is the major economic as well as health concern. The larvicidal potential of *O. limbata* plant was investigated against the 3rd instars larvae of *D. melanogaster*. The plant extract has tremendous potential for insect control as an alternative of artificial insecticides which can cause various adverse effects on the biology of earth. The death rate of 3rd instar larvae *D. melanogaster* feeding for 24 h on the medium with plus without (control) extract at 6% concentration of hexane extract was 90% by LC_{50} at 4.5%. The decline in dose concentration upshot in low larval mortality such as at 5% mortality reduced to 68% and at 4% reduced to 44%. So decrease in mortality was scrutinized with decrease in dose concentration[41]. The inhibitory potentials of the extract show the opposed effect of plant on pupal growth as larvae failed to develop into pupae after exposure to the extract. More or less all the mortality was recorded at larval stages. So this plant ultimately can be used for the control of many harmful insect pests of economical importance such as mosquitoes and houseflies which ground many serious diseases to human being plus animals[31]. The larvicidal activity of plant *O. limbata* is due to the presence of compounds such as tricyclic cisclerodane type diterpenoids well-known as limbatolide, beta-sitosterol and oleanolic acid[42]. The essential oils and extracts of *O. limbata* have noticable potential in the production of novel safe and ecofriendly larvicides and adulticidal compounds. These compounds possess very low risk to

human health. Products obtained from *O. limbata* extract contribute significantly to the diminution of environmental hazards as well as result in overall reduction of population density of some insect pests of fruits and many others vectoring pathogens of several human disease[41].

8. Toxicity profile

O. limbata did not exhibit any symptoms of unwanted side effects, acute neuro-impairment/toxicity at specific time period. The examination of neural impairment and acute toxicity after administration of *O. limbata* possess more safety profile, tolerability, therapeutic range as compared to the currently available antitussive agent such as dextromethorphan and codeine[43]. Subcutaneously administration plant extract yielded a significantly enhanced safety as compared to codeine and dextromethorphan administered by similar route of drug administration[36].

9. Chemical constituents

There are various constituents of the plant which are used in pharmaceutical industry, and they are marked as definable physiology and upon which therapeutic potential of the body is dependent. The action of these ingredients upon the body is investigated for therapeutic potential. There is no enormous interest regarding to its medical herbalist, because recently there is trend of using whole plant for whole people[44]. Depending upon chemical ingredients and isolation from plant does not tell us much about the activity of plant. It does not refute the study of such ingredients as it takes place highly holistic therapeutic approaches[36]. Classification of active ingredients depending upon physiologically is based on chemical structure rather than their specific action.

9.1. Limbatolide A, B, C, D

Limbatolide-A is gummy solid with $C_{21}H_{28}O_5$ molecular formula of compound checked from accurate mass measurement. The stereochemistry of the compounds with the evident of spectral data reveals the presence of the clerodane diterpenoids. *Cis-clerodanes* evident from chemical shifts C19 and C20 methyl carbon and while C17 and C20 methyl are trans to C19[45]. Limbatolide-B *cis-clerodane* tricyclic diterpene is gummy solid with $C_{21}H_{30}O_5$ molecular formula deduced from accurate mass measurement[46]. The stereochemistry shows H10 amplify the H3-19 intensity reveals with *cis-clerodanes*, methyls C17 and C20 reveals that trans to methyl C19. Limbatolide-C tricyclic diterpene *cis-clerodane* is also a gummy solid with molecular formula $C_{20}H_{28}O_4$ derived by accurate mass measurement[31]. Limbatolide D (molecular formula: $C_{20}H_{26}O_3$) is gummy solid and its NMR spectrum reveals the existence of trans-clerodanes five quaternary C-atoms, seven CH, five CH_2, and three CH_3[47]. CH_3-19 and H6 C *cis* relationship and b-configuration and also axial conformation of lactone at C6[48].

9.2. Limbatolide E, F and G

Limbatolide-E is another gummy solid and molecular formula is $C_{20}H_{26}O_4$[47]. The infrared spectrum illustrates the presence of an α,β-unsaturated lactone, OH group, furan ring and unsaturation. The ^{13}C-NMR confirms the presence of three CH_3 groups, four CH_2 groups, eight CH groups and five quaternary carbons in the compound. Further corroboration of the structure of compound is through nuclear overhauser effects ascertain, the spatial proximity of H2 and H10 and the OH group was present at C2 equatorially α-oriented[48]. Limbatolide-F and limbatolide-G compounds are trans-clerodane diterpenoids and both of them contain C4 and C6 α,β-unsaturated lactone[49].

9.3. Limbatenolide A, B, C and D

Limbatenolides is a gummy solid compound and has molecular formula of compound $C_{20}H_{22}O_4$ authorized by 1H- and by ^{13}C-NMR spectra. Existence of furan ring is confirmed in compound by UV spectra and infrared spectra. The ^{1}H-NMR reveals tetracyclic clerodane compound skeleton and 5-member lactone ring[50].

9.4. Limbatenolide-E, eupatorin and 3'-O-methyl eupatorin

Limbatenolide-E is another gum like solid constituent having molecular formula $C_{20}H_{22}O_4$ which belongs to class tetracyclic diterpenoids. Furan ring is not present in the compound that showed by UV spectra[50]. The eupatorin plus 3-O-methyl eupatorin 5,3-dihydroxy-6,7,4-trimethoxy-flavone compounds with molecular formula $C_{18}H_{16}O_7$ present in *O. limbata*. The structure of compound is stabilized through intermolecular and intramolecular hydrogen bondings between carbonyl O atoms and hydroxyl H atom, in addition to O-O interactions of 2.673 and 2.594 Å[3].

9.5. Ballotenic and ballodiolic acid

Ballotenic acid is colorless oil with molecular formula $C_{20}H_{30}O_4$ derived from mass measurement. The 1H-NMR spectrum revealed that ballotenic acid is a tricyclic clerodane and ^{13}C-NMR spectroscopy which illustrates the presence of two olefinic carbons, two tertiary methyl carbons, five quaternary carbons and one secondary methyl carbon[32]. A bicyclic and ballodiolic acid clerodane diterpene with $C_{20}H_{34}O_4$ molecular formula draw from accurate mass measurement. Ballodiolic acid had no five membered free lactone ring together with C12. The UV spectrum

demonstrates absorption band at maximal length 214 nm while broad peak appears at 2 926 cm^{-1} showing OH moiety of unsaturated carboxylicacid[32].

9.6. Kaempferol

Compound kaempferol-1 is a gummy solid and pale yellow in color and molecular formula of kaempferol-1 is $C_{45}H_{60}O_{29}$. According to ^{13}C-NMR, spectrum glycosylation is indicated at C3 and C7 position of kaempferol-1[2]. Compound kaempferol-2 is pale yellow and gummy solid while molecular formula of copmpund is $C_{54}H_{66}O_{31}$. The 1H- and ^{13}C-NMR spectra explains that kaempferol-2 has pentaglycoside as well as five anomeric H-atom, aglycone structure, two rhamnopyranosyl and three glucopyranosyl analyzed by 1D TOCSY and COSY spectral line[51]. Compound kaempferol-3 present in *O. limbata* is pale yellow and gummy solid. The molecular formula of kaempferol-3 of *O. limbata* is $C_{54}H_{66}O_{31}$[2]. Kaempferol-4 compound sperated from *O. limbata* is gummy solid and pale yellow in color and molecular formula of compound is $C_{63}H_{72}O_{33}$. The infrared spectral line of kaempferol-4 is evident for absorption bands of O-glycosidic linkage, α,β-unsaturated CO groups, OH groups, aromatic moieties and C-H stretching vibrations[51].

9.7. Others

Flavonoid p-coumaroyl glucosides are widely and siginificantly present in *Otostegia*. The isolation of such active compounds are considered to be very useful for not only the authentication but also for standardization of extracts particulary of those associated with medicinaly imperative[51].

10. Chemotaxonomic significance

Some genera of the Lamiaceae family commonly have flavonoid 4-hydroxy (E)-cinnamoyl glucosides and these are regarded as valuable markers for a chemotaxonomic point of view. The compound isolation has a tremendous value as kaempferol pentaglycosides are found in genus *Otostegia*. These compounds are quite useful for the authentication and standardization of medicinal interest as well as possess great chemotaxonomic value[51].

Conflict of interest statement

We declare that we have no conflict of interest.

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