ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 2 Issue 1

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

Antibacterial Activity of Medicinally Important Two Species of *Allophylus*- *Allophylus cobbe* (L.) Raeusch. and *Allophylus serratus* (Roxb.) Kurz.

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Allophylus (L.) (family Sapindaceae) has a ethnophramacological background and medicinally important genus.The two species of *Allophylus*- *A.cobbe* (L.) Raeusch. and *A.serratus* Roxb. (Kurz.) are used by local inhabitants in India against bone fractures, dislocations, wounds, cuts, ulcers and diarrhoea. In the present investigation, terpenoids and saponins are found accumulated in leaves of both species. *A.cobbe* shows higher level of terpenoids and saponins than *A. serratus*. Young and mature leaves of *A.cobbe* and *A.serratus* were evaluated for their antibacterial potential against *Bacillus subtilis* and *Staphylococcus aureus*. The aqueous and ethanolic extracts of young and mature leaves of *A.cobbe* and *A. serratus* exhibit good antibacterial potential against *B.subtilis* as compared to that of Cefotaxime. Thus, the leaves of *A.cobbe* and *A. serratus* may prove beneficial as a natural antibiotic against bacterial infections.

Keyword: Antibacterial activity, *Allophylus cobbe*, *Allophylus serratus*, *B.subtilis, S.aureus*

1. Introduction:

Allophylus is an important genus of the family Sapindaceae, found to grow on upline edges of hills in Western Ghats as well as mangrove associate at West Coast of India. Various species of *Allophylus* carry strong ethnopharmacological background. In Maharastra, the two species of this genus, namely *A.cobbe* (L.) Raeusch, and *A. serratus* (Roxb.) Kurz. Occur[1]. These two species are useful in traditional medical system and and carry strong ethnopharmacological background. *A. serratus* is used against bone fractures[2]. Leaves of *A.cobbe* are used by local people against bone fractures[3], rashes, stomach ache[4] and cuts and wounds[5].

Terpenoids are the largest group of plant products abundantly found in mangroves and are soluble in non-polar solvents. Terpenoids are the important class of natural products present in the form of volatile essential oils, triterpenoids, steroids and carotenoids[6]. Over 22,000 individual compounds of this class have been described[7]. They play diverse functional roles in plants as hormones, photosynthetic pigments, and electron carriers, mediators of polysaccharide assembly and structural components of membranes[8].

Saponins are water soluble and form the persistent foam upon shaking[9]. Due to this property, saponins containing plants are used as household detergents[10]. Saponins from plant origin have very interesting properties like spermicidal[11], molluscicidal[12], antibacterial and anti-inflammatory[13].

Bacillus subtilis produces extracellular toxin known as subtilisin which is having low toxicogenicity[14] which causes allergic reactions and hypersensitivity in individuals who are repeatedly exposed to it[15]. In animals, diseases by these bacteria reported are endocarditis, fatal pneumonia, bactaraemia in leukemic patients[16] , septicaemia (in patients with metastasising carcinoma of the breast, necrotic axillary tumour infections, another breast cancer patients, pleural effusions and breast prosthesis)[17]. *Staphylococcus aureus* causes abscess formation, supressuration, and variety of infections and fatal septicemia in human beings[18]. It causes respiratory tract infections[19], food borne illness and spoilage of food products[20].

In recent days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease[21]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions[21]. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents[21,22,23,24]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants[25].

As the leaves of *A.cobbe* and *A.serratus* are used by people to cure bone fractures, ulcers, cuts and wounds, in present study we have made an attempt to analyse phytochemicals and evaluate antibacterial activity of young and mature leaves of two species.

2. Material and Methods

2.1 Extraction of Plant Material

5g of dry powders of young and mature leaves of *A.serratus* and *A.cobbe* were taken and extracted in ethanol by using soxhlet extraction method. Then solvent was evaporated to dryness, giving a residue, which was dissolved in ethanol and used for antibacterial assay.

A. Test for Terpenoids

Salkowski test was applied for testing the presence of terpenoids. In 2 ml of aqueous extract, 2 ml chloroform was added followed by addition of conc. H_2SO_4. The reddish brown colour at the interface was the indication of presence of terpenoids[26].

B. Test for Saponins

1g dried powdered samples were taken in which 20 ml of distilled water was added. The mixture was boiled for a while, cooled to room temperature and filtered. Formation of red precipitate found presence of saponins in extract. 10 ml of filtrate and 5 ml distilled water were taken together and shaken vigorously. In that, 4 drops of olive oil was added and shaken vigorously. Formation of emulsion indicated the presence of saponins[26].

C. Antibacterial Assay

The ingredients (Peptone-2.5g, Beef extract-1.5g, NaCl-2.5g and Agar-8g) were dissolved in distilled water and pH was adjusted to 7 and volume was made to 1000ml with distilled water and autoclaved at 121°c and 15lbs for 15 min. The standard method described in The Indian Pharmacopoea was followed for screening antimicrobial activity[27] using agar well diffusion method by using sterile cork borer of size 7 mm. Wells were made in nutrient agar plate and inoculated with a loopful bacterial culture. 25 µL extract (of young and mature leaves) was poured in the well. Control used was Cefotaxime at it recommended dose for study of comparative efficacy. The plates after inoculation were transferred to BOD incubator set at 37°c. After 24 hrs, zone of inhibition around the wells was measured. Experiment was performed in replicates and data was statistically analyzed.

3. Results and Discussion

Table 1 exhibits results of qualitative tests for terpenoids and saponins in young and mature leaves, stem and root of *A. serratus* and *A. cobbe.* Test for terpenoid is negative in stem tissue of *A. serratus* and leaf tissue exhibits lower concentration of terpenoids and root tissue shows accumulation of terpenoids in *A. serratus*. Root, stem and leaf tissue of *A.cobbe* exhibits accumulation of terpenoids. Terpenoids exhibit properties like anti-inflammatory[28], antibacterial[29], antifungal[30], antiviral[31,32] ,antitumor[32,33]. Petalostemulol, a terpenoid from the ethanol soluble fraction of purple prairie clover showed strong activity against bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* and activity against gram negative bacteria and *Candida albicans*[34], (Hufford *et al.*, 1993).

Table 1: Qualitative screening of terpenoids and saponins in *A. cobbe* and *A. serratus*

Sample	Terpenoids	Saponins
YLAS	++	+++
MLAS	+	+
YSAS	-	++
MSAS	-	+
RTAS	+++	++
YLAC	+	+++
MLAC	+++	+++
YSAC	++	+
MSAC	+++	++
RTAC	++	+

Abbrv. : + Low concentration, ++ Moderate concentration, +++ High concentration, - Absent, YLAS-Young leaves of *A. serratus,* MLAS- Mature leaves of *A. serratus,* YSAS-Young stem of *A. serratus,* MSAS- Mature stem of *A. serratus,* RtAS-Root of *A. serratus*; YLAC-Young leaves of *A. cobbe,* MLAC- Mature leaves of *A. cobbe,* YSAC- Young stem of *A. cobbe,* MSAC-Mature stem of *A. cobbe,* RtAC-Root of *A. cobbe.*

Batista *et al.* (1994) isolated two diterpenes which worked well against bacteria like *Staphylococcus aureus, V. cholera, P. aeruginosa* and *Candida spp.* In present investigation, we noticed that terpenoid content in young parts of *A. serratus* and *A.cobbe* shows higher concentration while the roots of *A.cobbe* also indicates the moderate levels of terpenoids. The terpenoids indicate strong antifungal and antibacterial activity in various plant parts reported by several workers. Thus, the *Allophylus* leaves and stem with higher levels of terpenoids may serve as material to develop various antifungal, antibacterial biomolecules in near future.

Saponins are detected in all plant parts of *A. serratus* and *A.cobbe*. Saponins are significantly present in young leaves of *A. serratus* and both young and mature leaves of *A.cobbe* and are moderately detected from stem and root tissue of *A. serratus* and *A.cobbe.* Killeen *et al.* (1998) have reported antimicrobial activity of saponins. Li *et al.* (1999) isolated three jujubogenin saponins from *Colubrina retusa* (L) (Rhamnaceae) exhibits antifungal activity against *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*. According to Apers *et al.* (2000), tri terpenoid saponins isolated from *Maesa laceolata* Forssk (Myrsinaceae) possess structure activity relationships against dermatophytes HSV-1 and HIV viruses. BHMA (1983) reported that *Smilax ornata* and *Smilax regelii* contain saponins like sarsaponin and parallin and yield isomeric sapogenins, sarsapogenin and smilogenin. These species also contain sitosterol and stigmasterol in the free form and as glucosides. It is specific for psoriasis where there is desquamation.

In general, immature plants of a species have been found to have higher saponin contents than more mature plants of the same species (Francis *et al.*, 2002). We have reported saponins from leaves and stem of *A.cobbe* and *A. serratus* with higher intensity and the content of saponin is higher in immature parts than mature parts as indicated by Francis *et al*. (2002). In the present study, the saponin content is higher in young tender parts than the mature parts. The saponin exhibits haemolytic, antifungal, molluscicidal activity as well as it contributes as an important constituent in various herbal drugs and folk medicines exhibiting pharmacological properties as indicated by Estrada *et al*. (2000).
Thus, the *Allophylus* leaf and stem material might be useful in preparation of various types of herbal drugs.

The effect of aqueous and ethanolic extracts of young and mature leaves of *A.cobbe* and *A. serratus* against the *Bacillus subtilis* and *Staphyllococcus aureus* is shown in the Table 2. It is observed that Cefotaxime shows 14.75 mm zone of inhibition while the aqueous and ethanolic extracts of mature leaves of *A. serratus* show 19mm and 17mm maximum zone of inhibition, higher than the young and mature leaves of *A.cobbe.* It is observed that the young and mature leaves of *A.cobbe* and *A. serratus* exhibits greater zone of inhibition than the

Cefotaxime against the gram positive bacterium *B. subtilis.*

Table 2: Effect of aqueous and ethanolic extracts of *A. serratus* and *A.cobbe* on bacterial growth, *viz. Staphylococcus aureus* and *Bacilus subtilis.*

Bacteria	Sample	Zone of inhibition in mm				
		A. serratus		*A.cobbe*		Cefotaxime
		YL	ML	YL	ML	
Staphylococcus aureus	Aqueous	17.7 ±3.4	17.3 ±5.5	18.3 ±3.9	20.3 ±2.6	31.58± 1.2
	Ethanolic	20.7 ±5.0	18±3 .8	21±6 .8	23.7 ±4.1	
Bacilus subtilis	Aqueous	19±5 .5	19.3 ±4.1	19.3 ±2.0	17±4 .8	14.75± 3.2
	Ethanolic	17.7 ±3.4	17.3 ±5.5	18.3 ±3.9	20.3 ±2.6	

Abbrv. YL- Young leaves, ML-Mature leaves

As considered activity against *Staphyllococcus aureus,* it is observed that the standard antibiotic Cefotaxime exhibits zone of inhibition against *S. aureus* while the aqueous and ethanolic extracts of young and mature leaves of *A. serratus* and *A.cobbe* shows the maximum zone of inhibition in the range of 20-23mm which is comparatively less than standard antibiotic. Parekh *et al.* (2006) screened antibacterial potentiality of some traditionally used medicinal plants viz. *Abutilon indicum* L., *Acorous calamus* L., *Ammania baccifera* L., *Argyrea nervosa* Burm. F., *Bauhinia variegata* L., *Crataeva religiosa* Forst., *Hedychium spicatum* L., *Holarrhena antidysentrica* L., *Piper nigrum* L., *Plumbago zeylanica* L., *Psoralea corylifolia* L. and *Saussurea lappa* Costus. They found that *B. variegata* was most powerful among all plants tested against *E.coli*, *K. pneumoniae, S. aureus, B. cereus and P. pseudoalcaligenes.* Aqueous extracts were found less effective as compared to methanolic extracts except *P. nigrum* and noticed inhibition zones against *S. aureus* were in range of 0-20 mm. Latha and Kannabirian (2006) investigated antimicrobial activity of extracts of *Solanum trilobatum* L. plant parts like leaves, flowers, stem and fruits in which methanol extract of stem showed the maximum inhibition against *S. aureus* (11mm) while aqueous and n-butanol extracts of leaves showed zone of inhibition 7mm and 5mm respectively. Shinde *et al*. (2007) noticed that the inhibition of the growth occur in the presence of 1% leaf extract of *Andrographis paniculata* highest in case of *Staphylococcus aureus* followed by *E. coli* and *Bacillus substilis.* Dar *et al.*(2008) investigated antimicrobial activity of different extracts of *Withania somnifera* against various pathogenic bacteria. They observed that at even 25% concentration of benzene extract of *Withania somnifera,* there was total inhibition of the growth of *Staphylococcus aureus* (Inhibition Zone -8 mm). Mudi and Ibrahim (2008) tested ethanolic extracts of leaves of *Bryophllum pinnatum* S. Kurz. against *Staphyllococcus aureus* and they found its effectiveness showing zone of inhibition 12 mm. Rondon *et al.* (2008) evaluated phytochemical content and antibacterial activity of aerial parts of *Porophyllum ruderale* (Jacq.) Cass. collected in Venezuela. They observed that minimum inhibitory concentration of essential oil for effective antibacterial activity (against *S. aureus*) was 20μg/ml and zone of inhibition was 19mm against *Staphyllococcus aureus.* Antibacterial potential of leaf essential oils of *Eucalyptus camaldulensis* and *Myrtus communis* L. growing in Northern Cyprus was evaluated by Akin *et al*. (2010). MIC of *E. camaldulensis* was 0.5 % against *S. aureus* and >1% against *B. subtilis* while it was 0.5% of *M. communis* against *S. aureus* and *B. subtilis.* Bukar *et al.,* (2010) evaluated leaf and seed extracts of *Moringa oleifera* Lam. against some food borne microorganisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*. They found presence of alkaloids, flavonoids while saponins in seed ethanolic extracts and absence of tannins while ethanolic extracts of leaves contained flavonoids and saponins while absence of tannins and alkaloids. 400mg/ml ethanolic extracts of seeds indicated zone of inhibition 11mm and MIC was more than 4mg/ml while leaf ethanolic extracts (400mg/ml) showed zone of inhibition against *S. aureus* as 9mm and MIC was 2mg/ml.

In recent days, making antibacterial drug therapy effective, affordable and safe has been the focus of interest (Sharma *et al.*, 2002). Various researchers have reported about antibacterial activity of medicinal plants (Adelakun *et al.*, 2001, de Boer, 2005, Nair *et al.*, 2005, Joshi *et al.*, 2009). Considering the above aspects, in present investigation, attempt has been made to find out preliminary antibacterial activity of different plant parts of *A.cobbe* and *A. serratus*. The object of this study is to select an active plant extract which may be useful in developing new drug targets to combat deadly bacterial diseases. In the present study, aqueous and ethanolic extracts of young and mature leaves of *A.cobbe* and *A. serratus* exhibit good antibacterial potential against *B. subtilis* than *S. aureus*. Thus, the extracts of *Allophylus* leaves can be implicated to control the gram positive endospore forming bacterium *B. subtilis.*

4. Conclusion

It is concluded that, the results obtained in the present study lend support to a certain extent with the use of the *A. serratus* and *A.cobbe* in traditional medicine. The obtained results could form a good basis for selection of plant species and their parts for further investigation in the discovery of novel bioactive compounds.

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