Studies on Antibacterial Activity of Acmella oleracea (L.) Murr.

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Abstract:

Acmella oleracea (L.) Murr (synonym Spilanthes oleracea L. (1767), Spilanthes acmella auct. non (L.) Murr) is a small herb belonging to family Compositae. The most common and widespread traditional medicinal use of this plant is to treat toothache and throat & gum infections. The flowerheads are used either fresh or dried & powdered, but the use of roots and leaves has been recommended as well. The plant is further recommended as a cure for dysentery and rheumatism, and to enhance the immune system. It is used against blood parasites, especially against malaria, both prophylactic and curative.

In present study, the effect of leaf and inflorescence extracts are tested on eight different bacteria viz. Bacillus megaterium, B. subtilis, B. cereus, Escherichia coli, Klebsiella pneumonaie, Micrococcus luteus, Nocardia sp. and Pseudomonas aeruginosa. After 24 hours incubation it is observed that the plant extracts have good inhibitory action against the bacteria except Nocardia sp.

Key words: Acmella oleracea, Compositae, toothache plant, spilanthol, antibacterial activity,

Introduction:

Plants are oldest source of pharmacologically active compounds and have provided many medicinally useful compounds from centuries to the mankind. Today it is estimated that more than two third of the world's population relies on plant-derived drugs. The research for natural products to cure diseases represents an area of great interest in which plants have been the most important source.

The Compositae (Aster) constitutes one of the largest vascular plants' family with 30,000 species and over 1100 genera. The genus Acmella contains 35 tropical species of which three are reported from India (Rani et. al. 2006). Most of these plants exhibit

antimicrobial activity due to the production of a sesquiterpene as principal secondary metabolites.

Acmella oleracea (L.) Murr (synonym: Spilanthes oleracea L. (1767), Spilanthes acmella auct. non (L.) Murr) is a small herb belonging to family Compositae (para-cress or toothache plant) (Chopra et. al. 1956). It is unique and versatile plant that will add texture and interest to the garden. The compact pompom shaped flowerheads that are set atop of elongated stem, begin as squat affairs, gradually elongate and turn yellow retaining the red portion at the top. The leaves are dark green in colour. The name toothache plant comes from numbing property it produces when the leaves and flowers are chewed (Jansen 1985). The plant has long been used in India for the treatment of gum & dental problems, rheumatism and fever. The leaves and flower heads have analgesic, antifungal, antihelminthic, antibacterial immunomodulatory, adaptogenic, lithotriptic, antiscorbitic, sialagogine and digestive property (Savadi et. al. 2010). The plant also has Vasorelaxant and Antioxidant (Wongsawatkul et. al. 2008) and local anesthetic and antipyretic activities (Chakraborty, et. al, 2010) due to presence of spilanthol (Prachayasittikul 2013).

The present study was aimed to evaluate the activity of flowerheads and leaf extracts of A. oleracea (L.) Murr (Asteraceae) against potential pathogens viz. Bacillus megaterium, B. subtilis, B. cereus, Escherichia coli, Klebsiella pneumonaie, Micrococcus luteus, Nocardia sp. and Pseudomonas aeruginosa.

Materials and Methods:

a) The Plant material:

Fresh plant parts viz. leaves and flowerheads of A. oleracea (Photo plate No. 1) are collected from the kitchen garden of an Ayurvedic Doctor in Chiplun town of Ratnagiri district (Maharashra) in the month of November. The plant parts were washed thoroughly in running tap water, rinsed with distilled water, dissected in to pieces, air-dried at room temperature, powdered and stored as stock powder in airtight amber coloured bottles for further use.

b) Solvent extraction of active compounds:

Five gram stock powder of each of the plant portions was mixed with 50 ml organic solvents (Methanol, Hexane and Chloroform) separately in a conical flasks and after plugging, the material was kept shaking at 190 - 220 rpm on Remi make metabolic shaker for 24 hrs. After 24 hrs, the mixtures were filtered through 8 layered muslin cloth filter and

centrifuged at 5000 rpm for 15 min. the supernatants were collected and the solvents were evaporated to make the final volume one-fourth of the original. Then the extracts were stored at 4 ^{o}C in airtight bottles for further use.

c) Test Microorganisms:

The bacterial cultures used in the study were Bacillus megaterium, B. subtilis, B. cereus, Escherichia coli, Klebsiella pneumonaie, Micrococcus luteus, Nocardia sp. and Pseudomonas aeruginosa. These bacteria were procured from Dept. of Microbiology, NCL, Pune (Maharashtra). The cultures were maintained on nutrient agar slants at 37ºC for about 18-24 hours and then stored at 4ºC as stock cultures for further antibacterial activity. Fresh culture were obtained by inoculating loopfull of stock into nutrient broth and incubating at 37ºC overnight.

d) Antibacterial assay:

For antibacterial activity, 10 gm of digested nutrient agar medium and 90mm diameter Borosil make petridishes were autoclaved at 15 lbs pressure for 20 min. Agar cup bioassay technique was employed for testing the antibacterial activity of extracts. To each petriplate, 20 ml of sterile medium was added. After setting the agar, 0.1 ml of inoculum was added and spread uniformly using sterile glass spreader. After inoculation, cups were scooped out with the help of 7 mm sterile cork borer and to each cup, 100 µl of different concentrations (1%, 3%, 5% and 10%) of test solutions prepared in different solvents (Chloroform, Hexane and Methanol) were added. Appropriate controls were also prepared using respective solvents. Inoculated petridishes were incubated at 37 ^{o}C for 24 hrs and antibacterial activities were recorded.

Results and Discussion:

It was observed that only methanol extracts of leaves and flowerheads of Acmella oleracea (L.) Murr (Photo Plate No. 1) showed zone of inhibitions and no zone of inhibition were seen for Chloroform and Hexane extracts. The results of methanol extracts are presented in table no. 1 & 2.

Acmella oleracea (L.) Murr (synonym: Spilanthes oleracea L. (1767), Spilanthes acmella auct. non (L.) Murr) is a small herb belonging to family Compositae (para-cress or toothache plant) (Chopra et. al. 1956). The name toothache plant comes from numbing property it produces when the leaves and flowers are chewed (Jansen 1985). The plant has long been used in India for the treatment of gum & dental problems, fever and rheumatism.

The flower heads contain analgesic, antifungal, antihelminthic, and antibacterial agents and the leaves are used as immunomodulatory, adaptogenic, toothpaste, lithotriptic, antiscorbitic, sialagogine and digestive (Savadi et. al. 2010). The plant has Vasorelaxant and Antioxidant Activity (Wongsawatkul et. al. 2008), anesthetic and antipyretic activities (Chakraborty, et.al, 2010). The plant contains a sesqueterpene, spilanthol as a major secondary metabolite along with several other organic compounds (Prachayasittikul) 2013.

The results depicted in present study indicate that the methanol extracts of flowerheads and leaves have good antibacterial activity against the tested eight important bacteria. Similar kind of antibacterial activities are also reported by Thompson et al, (2012) in Pseudomonas aeruginosa, Streptococcus salivarius, Streptococcus viridans, Streptococcus mutans, Bacillus megaterium and Neisseria catarrhalis. These bacteria are reported to be concerned with orodontal problems in human beings. Different types of phytochemicals present in the extracts have different mode of action on the various test bacteria (Rizvi et. al., 2011). In present study, differences are observed in susceptibility of various test bacteria towards the extract. These differences could be attributed to the nature of phytochemicals present in the extract.

Conclusion:

From the above results it can be concluded that the methanol extracts of flowerheads and leaves of A. oleracea have good antibacterial activity against the tested organisms. Hence the use of such extract may be promoted in traditional herbal remedy system to cure the infections caused by tested organisms.

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Table No. 1. Effect of methanol extract of leaves of A. oleracea.

Organisms	Zone of inhibition in mm. for various concentrations				
	Control	1%	3%	5%	10%
Bacillus megaterium	0.00	04	07	13	18
B. subtilis	0.00	02	13	16	18
B. cereus	0.00	01	03	04	04
E. coli	0.00	03	14	18	16
Klebsiella pneumonaie	0.00	05	12	19	21
Micrococcus luteus	0.00	02	06	10	17
Nocardia sp.	0.00	01	01	01	01
Pseudomonas aeruginosa	0.00	03	05	07	09

Table No. 2. Effect of methanol extract of Flowerhead of A. oleracea..

Organisms	Zone of inhibition in mm. for various concentrations				
	Control	1%	3%	5%	10%
Bacillus megaterium	0.00	04	06	12	17
B. subtilis	0.00	03	09	16	20
B. cereus	0.00	02	09	16	19
E. coli	0.00	04	12	17	19
Klebsiella pneumonaie	0.00	04	13	18	22
Micrococcus luteus	0.00	02	05	11	19
Nocardia sp.	0.00	01	01	01	01
Pseudomonas aeruginosa	0.00	03	06	09	11

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Photo Plate No. 1. Acmella oleracea (L.) Murr.

A) A. oleracea Young Plant.



B) A. oleracea Plant with flowerheads.

