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Analgesic and Anti Microbial Activity of Aerial Parts of *Polygonum Glabraum*

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Abstract

The Methanol and Petroleum ether extract of the aerial parts of *polygonum glabraum* was investigated for its analgesic activity in animal models. The methanol and pet ether extract at 200 mg/kg body weight in the acetic acid induced writhing model, the extract showed a good analgesic effect characterized by reduction in the number of writhes when compared to control. The both extract were screened for their antibacterial activity by filter paper disc method. The extracts were tested against *Staphylocccusaureus* and *Pseudomonas aureginosa*and fungi like *Candida albicans*.The susceptibility of the microorganisms to the extracts of the leaves of plant was compared with selected standard antibiotics (Amikacin 5µgm/ml) and Ketoconazole (10 µgm/ml). The methanol extract the zone of inhibition is 15 mm in S. aureus and 12 mm in P.auregenosa for anti bacterial activity. The antifungal activity against candida albicans in methanolic extract is 11mm.

Keywords*: Polygonumglabraum,, Analgesic activity, Antibacterial activity*

Introduction

Pain is the local swelling compresses sensory nerve ending. The tissue injury causes the release of chemicalmediators which activates nociceptor. The prostaglandins, leucotriens and thromboxanes are release on the site of injury as chemical mediators. Cyclooxygenase is the key enzyme which catalyses the conversion arachidonic acid to prostaglandins and thromboxanes. There are two types of cyclooxygenase enzyme, COX-1and COX-2. COX-1 is constitutive enzyme and COX2 is inducible which produce inflammation[1]. Non-steroidalanti-inflammatory drugs (NSAIDS) widely used in the treatment of pain and inflammation. These compounds non selectively inhibit the two iso form of cyclooxygenase enzymes and thus prevent the metabolism of arachidonic acid and upgration of prostaglandin production[2, 3].

In recent years there has been a growing interest to evaluate plants possessing antimicrobial activities,for various disease[4].A number of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants [5-7]. Plant derived drugs have become a popular alternative medicine in developing countries. Synthetic antifungal/antibacterial drugs widely used at present are sometimes causing toxicity and adverse drug reactions[8]. Furthermore, herbal medicines and supplementation are considered less toxic than the synthetic compounds[9]. *Polygonumglabrum*commanoly known as polygonum in English, Atalari in Tamil. Presence of carbohydrate, glycosides, alkaloids, flavonoids, flavones, Phenolic compounds, Protein,aminoacids, Tanins and saponins has been reported in the plant. The plant is described as anthelmintic[10], anti malarial[11], anti fungal[12], anticancer[13], anti bacterial[14], anti pyretic[15],anti inflammatory[16], anti tumor[17] and diuretic[18]. The root stocks are used in piles, jaundice and debility. Seeds are used in leucorrhoea. Stem peels are burnt over affected parts of the body to treat rheumatism. Plant pacifies vitiated pitta, inflammation, cough, asthma, pneumonia and liver and spleen diseases.

Materials and Methods

Plant material

The aerial parts of *Polygonumglabarum* Wild were collected from Madurai during the month of July and identified by Dr.Stephen , American College, Madurai.

Preparation of the extract

The leaves and stem bark were cut off and dried in shade for 45 days. Then about 3 kg of the shade dried

leaves and stem bark was made in to coarse granules. Dried powder material of *Polygonumglabraum*were defatted with petroleum ether in a soxhlet apparatus. The defatted powder material was obtained and further extracted with methanol for 72 hrs in soxhlet apparatus. The resulting semisolid mass was dried and used for phyto chemical analysis and hyper glycemic activity. Preliminary phyto chemical investigation[19-22] was carried out and results are tabulated in table 1.

Animals

The animals used in this study were male Wister rats weighing between 20 and 25g. They were maintained at the experimental animal house of the agricultural and rural development Research institute.They were kept in rat cages and fed on commercial rabbit cubes.

Analgesic activity[23-27]

Treatment protocol

The group1 treated as normal control received 10ml/kg of normal saline through orally. The group2 treated as standard control received 10mg/kg of diclofenac sodium through intraperitonealy. The group3 treated as treatment control received 200mg/kg of petroleum ether extract of *polygonum glabrum* is spended with 2ml of 1% CMC administered through orally. The group4 treated as treatment control received 200mg/kg of methanolic extract of*polygonum glabrum* suspended with 2ml of 1% CMC administered through orally.

Both the extract was administered one hour prior to the acetic acid administration. Note the onset on writhing. Record the numbers of abdominal contractions, trunk twist and extension of hind limbs as well as the number of animals showing such response during a period of 10 minutes were noted Table 2.

Statistical analysis

Data are expressed as mean ± SEM; data analysed by one way ANOVA followed by Newman's keul's multiple range tests to determine the significance of the difference between the control group and rats treated with the extracts. Values were considered significant at $p<0.01$.

Antimicrobial activity[28-30]

Antibacterial activity

The sterilized Muller-Hinton agar media was heated on a waterbath to melt the media. When the media was luke warm, the organism was inoculated separately and poured aseptically into sterile petridishes and allowed to solidify. The standard drug Amikacin disc was placed on the media and the Whatmann No.2 filter disc (5mm diameter) were cut and filled into vials plugged with cotton. These vials were kept in hot air oven at 160°c for 30min for sterilisation. Then it was soaked in two extracts separately and evaporated to dryness and kept on the media (5mm height). One more disc immersed in methanol and kept on the media as control. It was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug and later kept in the incubator for a period of 24hours at 37°c. Observation was made for the zone of inhibition around the synthesized compounds with that of standard Table 3.

Anti-fungal activity

For the screening of antifungal activity disc diffusion method was used. Saboured dextrose agar plate were prepared aseptically to get a thickness of 5-6mm. Theplates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37°c, which show significant growth of fungi. The temperature of the medium should not exceed about 50°c when the organisms were inoculated. The standard drug Ketoconazole (10µm/disc) was placed on the media. The sterile Whatmann No. 2 filter paper disc (5mm diameter) was soaked in two extracts (20µm/disc) separately and evaporated to dryness and then kept on media. One more disc immersed in methanol and kept on the media as control. The petridishes were incubated at 37°c for 24hours, after placing them in refrigerator for 1 hour to facilitate uniform diffusion. Observations were for the zone of inhibition around the extracts and with that of standard Table 4.

Results and Discussion

Due to the increasing frequency of intake of NASAIDS and their reported common side effects there is need to focus on the scientific exploration of potential herbal drugs having fewer side effects. Here *polygonumglabraum* being an indigenous drug used by different communities for long is tried on experimental animals to assess the efficacy of the drug on analgesic activity. The petroleum ether extract does not possess significant analgesic activity whereasmethanolic extract possess significant analgesic activity at $p<0.01$. Disc diffusion methods are used extensively to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions with a low activity however a large concentration is needed. Due to the limited capacity of discs, holes or cylinders are preferably used. Microbiological Screening reveals that methanolic extract possesses significant anti-microbial activity when compared with petroleum ether extract.

Table: 1: Preliminary phytochemical screening of the various extracts of *polygonum glabraum wild*

Consituents	PEPG	MEPG
Carbohydrates	+	+
Glycosides	+	+
Alkaloids	-	+
Flavonoids	-	+
Flavones	-	+
Phenolic compounds	-	+
Tannins	-	-
Protein & Amino Acids	+	+

+ - Indicate positive test results.
- - Indicate negative test results.

These crude extract were also used for the exhibition of some selective pharmacological properties.

Table 2: Analgesic activity of various extracts of polygonum glabrum by acetic acid induced writhing reflux in mice

Treatment	Dose(mg/kg)	No. of writhing	%Reduction in reaction time
Group I Normal saline	Inject1%v/v acetic acid 1ml/100g of body weight	39.5±4.6	-
GroupII Std	10mg/ kg I.P Diclofenac sodium	6.4±1.22	83.79%**
GroupIII Pet. Ether extract	200mg/kg Administered through orally.	28.6±3.2	27.59%
GroupIV Methanolic extract	200mg/kg Administered through orally.	11.3±2.0	71.39%**

Values are expressed as mean ±SEM
Values were find out by using one-way ANOVA followed by Newman's keuls multiple range tests.
**Values were considered significant at P<0.01.

Table 3: Anti bacterial activity

A) Staphyloccus Aureus

S.NO	SAMPLE	ZONE OF INHIBITION
1.	Standard	17mm
2.	Methanolic extract	15mm
3.	Pet. Ether extract	4mm

B) Pseudomonas Aurogenosa

S.NO	SAMPLE	ZONE OF INHIBITION
1.	Standard	14mm
2.	Methanolic extract	12mm
3.	Pet.Ether extract	6mm

Table 4: Anti-fungal activity

Candida albicans

S.NO	SAMPLE	ZONE OF INHIBITION
1.	Standard	14mm
2.	Methanolic extract	11mm
3.	Pet.Ether extract	7mm

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