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PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF BARK EXTRACT OF ACACIA NILOTICA FOR ANTITUSSIVE SCREENING

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ABSTRACT

In the present work explore the folkloric reported cough suppressant activity of the bark of the *Acacia nilotica*. The macroscopic evaluation revealed yellow colored velvety flowers, bipinnate leaves with hairy axis and reddish brown bark. The microscopic evaluation of the transverse section of the bark revealed thick epidermis whereas the powder characteristics exhibited xylem vessels and starch granules. The results obtained from phytochemical testing of the successive solvent extracts of the barks of *Acacia nilotica* revealed the presence of alkaloids in the aqueous extracts. Phenols and tannins were obtained in the ethyl acetate, methanolic and aqueous extracts whereas flavonoids were obtained in the ethyl acetate extract and methanolic extract. On the basis of the qualitative phytochemical analysis results it was evident that the major portions of the phytochemicals especially tannins, saponins, flavonoids and triterpenoids were present in the ethyl acetate and methanolic extracts of the bark of *Acacia nilotica*. These two extracts were evaluated for antitussive action in mice using ammonium liquor induced cough and sulfur dioxide induced cough models at two different dose levels (250 and 500 mg/kg, po).The results indicate that the bark extract of *Acacia nilotica* demonstrated antitussive effect in *in vivo* experimental models by prolonging the latency period of coughing and also reducing the frequency of coughing bouts in mice. The increase in latency as well as the decrease in the frequency of coughing bouts occurred in a dose dependent fashion in both the extracts. It was obvious from the results that the antitussive action was much more significant in the methanolic extract as compared to the ethyl acetate extract. The dose of 500 mg/kg of the methanolic extract was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models.

Key-words: *Acacia nilotica*, Methanolic Extract, Antitussive Activity, Phytochemical Analysis.

INTRODUCTION

Plant and other natural products have been in use for the human sufferings from time immemorial. The search for new chemical entities obtained by screening natural sources such as plant extracts and microbial fermentation had led to the discovery of many clinically useful drugs that play major role in treatment of human diseases. Today, higher plants continue to retain their historical significance as important sources of novel compounds useful either directly as medicinal agents or as lead compounds for synthetic/semi synthetic structural modification/ optimization or biochemical/pharmacological probes. Natural products, in particular herbs, have been used for the treatment of various diseases for thousands of years. Terrestrial plants are used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs are developed from them. The primary written records on the medicative uses of plants appeared in concerning 2600 BC from the Sumerians and Akkaidians [1]. The "Ebers Papyrus", the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of

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Egyptian medicine dated from 1500 B.C. The Chinese Materia medica, which describes more than 600 medicinal plants, has been well documented with the first record dating from about 1100 B.C. [2]. Documentation of the

Ayurvedic system recorded in Susruta and Charaka dates from about 1000 BC [3]. The use of medicinal plants was compiled in Ayurveda, which listed more than 8000 herbal remedies. India is one of the world's twelve leading biodiversity centers with the presence of over 45,000 different plant species. Of these, about 15,000-20,000 plants have medicinal properties of which only about 7000-7500 are being used by traditional practitioners.

Anti-tussives are remedies that alleviate coughing. Some anti-tussives work by soothing irritability (respiratory demulcents); others are claimed to relieve coughs at source, by removing congestive mucus or other mobile provocations (expectorants). Although the centrally acting opioids still remain the antitussive drug of choice for decades, they possess side effects such as sedation and gastrointestinal symptoms. Therefore, there is a need to have safe and effective antitussive that can successfully alleviate chronic cough without developing side effects. In view of these inconveniences, the ailing population is turning toward certain alternatives for satisfactory remedies. In such scenario, Ayurveda can offer a number of drugs through its armamentarium. Researches of recent past have proven antitussive activity of many herbs.

In addition, certain compound herbal formulations of ayurveda such as *Vyaghri Haritaki*, Avaleha, Vasavaleha, Shirishavaleha, Sitopaladichoorna, and Shirisharista are reported to possess significant antitussive activity.

Acacia nilotica is an evergreen, usually small sized (2.5-25 m) tree with a short, thick and cylindrical trunk. Its bark is grey, reddish-brown or black, rough and furrowed. The leaves of the tree are alternate, bipinately compound, 5-15 cm long; with a hairy axis containing 3-8 pairs of side axes, grey-green in colour. Flowers are sweetly scented and bright to golden yellow in colour. The fruits are linear, flattened, narrow indehiscent pods, 4-22 cm long and 1-2 cm broad, dark-brown to grey in colour and glabrous or velvety. The pods contain 8 to 15 elliptical, flattened bean-shaped dark seeds.

The detailed study of the review of literature led to the conclusion that different parts of *Acacia nilotica* have been extensively used for their medicinal properties. A lot of activities reported in the folkloric medicine as well as Ayurveda have been scientifically explored by researchers' worldwide. It was also evident from the review that the bark of the plant was scientifically the least explored part. It was therefore envisioned to explore the folkloric reported cough suppressant activity of the bark of the plant.

EXPERIMENTAL

Collection of Plant Materials

Stem bark of the plant under investigation i.e., *Acacia nilotica* was collected from nearby areas of Bhopal (M.P), India in the month of May 2019. The taxonomical identification and authentication of the plant material was done at Saifia science college Bhopal (Affiliated to barkatullah university Bhopal, India). The specimen of plant sample has been submitted and preserved in the herbarium of the institute.

MATERIAL AND METHODS

Preparation of *Acacia nilotica* powder

The stem bark of *Acacia nilotica* was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve no. 40 and stored in a labeled air tight container for further studies.

Physicochemical Studies

Physicochemical studies include ash value and extractive value to determine the quality and purity of the powder of plant of *Acacia nilotica*.

Macroscopical Evaluation

Medicinal plant materials are categorized according to Organoleptic, microscopical and macroscopical characteristics. Taking into consideration the variations in source of crude drug and their chemical nature, they are standardized by using different techniques including the methods of estimation of chief active constituents. Organoleptic evaluation of drugs refers to the evaluation of drugs by color, odour, size, shape, taste and special features including touch and texture etc. Organoleptic evaluations can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity.

Microscopical Evaluation

Microscopical evaluation includes the study of transverse section of the bark observed under a microscope.

Extraction

Defatting of Plant Material

The powdered bark of *Acacia nilotica* was subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

Successive Solvent Extraction

The collected, cleaned powder of stem bark of *Acacia nilotica* was used for the extraction process. The powder of the plant (250 g) material was evenly packed in the soxhlet apparatus and extracted with various solvent increasing polarity including petroleum ether, chloroform, ethyl acetate, methanol by hot continuous extraction process for about 18 hr except aqueous, separately. The

aqueous extraction was carried out by cold maceration process after the solvent extraction process was complete. The extracts were filtered while hot through Whatman filter paper to remove any impurities if present. The extracts were concentrated by vacuum distillation to reduce the volume to 1/10. The concentrated extracts were transferred to 100 ml beaker and were evaporated on the water bath. The dried extracts was packed and labeled in air tight container for the further studies.

Preliminary Phytochemical Screening

The preliminary phytochemical studies were performed for testing different chemical groups present in the solvent extracts.

Pharmacological Study

The *in-vivo* antitussive activity was carried out in *Albino* mice of either sex weighing between 25–30 g by using sulphur dioxide induced cough method and ammonium liquor induced cough method. The protocol of the present work was approved by Institutional Animal Ethical Committee (IAEC), Technocrats Institute of Technology-Pharmacy, Bhopal, India. (Ref.no.TIT/IAEC/831/P'Col/2019/07). In the departmental animal house, the mice were group housed in poly acrylic cages (38x23x10 cm) with not more than four animals per cage and maintained under standard laboratory conditions with natural dark and light cycle (14 hr light/10 hr dark) at 27±2°C and relative humidity (RH) 44-56% with free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for one week before and during the experiments. The animals were allowed to acclimatize one week before the start of experimentation.

Animal were divided into 6 groups of 6 animals each for each protocol. Group I served as control and was administered with normal saline, group II, III, IV & V served as treatment groups and were administered with extract of *Acacia nilotica* whereas group VI served as positive control and was administered with codeine phosphate, 20 mg/kg p.o (Table 1).

Table 1. Grouping of Animal for Antitussive Screening

Experimental Group	Treatment	Dose (mg/kg, *p.o.*)	No. of Animal
Group I	Control	Normal control	6
Group II	Ethyl acetate Extract	250	6
Group III	Ethyl acetate Extract	500	6
Group IV	Methanolic Extract	250	6
Group V	Methanolic Extract	500	6
Group VI	Codeine phosphate	20	6

***In-vivo* Sulphur Dioxide Induced Coughing**

The *in-vivo* antitussive activity against sulphur dioxide (SO_2) induced cough was performed by the method described by Miyagoshi *et al.*, 1986. A 2 ml solution of 500mg/ml of sodium hydrogen sulfite (NaHSO3, SD Fine Chemicals, Pvt. Ltd.) in distilled water was placed in a vial at the base of a desiccator and covered with wire gauze to act as a platform for placement of mice. Concentrated sulphuric acid (H_2SO_4; CDH, New Delhi, India) was introduced into the vial to evolve sulphur dioxide gas.

$$2NaHSO_3 + H_2SO_4 \rightarrow 2SO_2 + Na_2SO_4 + 2H_2O$$

The mice were placed on the wire gauze platform in the desiccator after 15 seconds and exposed to SO_2 for 45 sec. The mice were removed from the desiccator and placed in an observation chamber for counting of cough bouts for 5 minutes thereafter. Similar procedure was repeated for all the mice of the treated groups and the frequency of cough bouts was measured. Frequencies of cough bouts (number of coughing) were counted by using stopwatch. Initially the animal of all groups were individually placed in the desiccator and the cough bout responses (zero min) were recorded. After 45 seconds exposure of gas, the animal was removed from the desiccator and observed the frequency of cough bout for 5 min. In the same way, the frequency of cough bout observed for all the animal groups at zero min before the administration of extract and at 1 hr after the administration of extract.

***In-vivo* Ammonium Activity Liquor Induced Cough**

The *in-vivo* antitussive activity against ammonium liquor (NH_3) induced cough was evaluated. Healthy mice were selected and divided into seven groups: After 1 hr of oral administration of test drug, individually all mice of each group was placed on wire gauze platform in desiccator and exposed to 0.3 ml NH_4OH (25%) vapours generated by a nebulizer for 45 second. After 45 second the mice were removed from the desiccator and placed in an observation chamber for monitoring of frequency of cough bouts for 5 minutes thereafter. The frequency of cough bouts was counted by using stopwatch.

Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Student's t-Test. The result were considered statistically significant when P- value less than 0.05 ($P<0.05$) vs. control.

RESULTS AND DISCUSSION

Macroscopic and Microscopic Evaluation

Organoleptic evaluation represents observing of those properties of materials for which sense organs can be

used. It thereby defines some specific characteristics of the material which can be considered as a first step towards establishing the identity and degree of purity of the material. The organoleptic parameters (color, odour and taste, and texture) were evaluated and are presented in Table 2.

Antitussive Screening of Extracts

The evaluation of antitussive activity in animal can be done by inducing cough by mechanical stimulus, electrical stimulus, and chemical stimulus. In the present study chemical induction of cough was performed and the effect of the bark extract on cough bouts was recorded.

A cough bout response to a given stimulus varies from animal to animal but fairly reproducible if we repeat the measurements within the same animals. So, low or high cough bout threshold in animals were not entertained for further studies. The frequency of cough bouts was observed for all animal groups at 1 hr after administration of standard drug as well as *Acacia nilotica* bark extracts. The percentage inhibition of frequency of cough bout was calculated by the formula-

% percentage inhibition of frequency of cough =

$$[(Ca - Ta) / Ca] \times 100$$

Where,

Ta= Frequency of cough bout in treated animal

Ca= Frequency of cough bout in control group

The time interval between exposure to ammonia hydroxide or SO_2 and appearance of cough bouts is called as the cough latency period. It is a measure of the potency and efficacy of the drug under study.

On the basis of the qualitative phytochemical analysis results it was evident that the major portions of the phytochemicals especially tannins, saponins, Flavonoids and Triterpenoids were present in the ethyl acetate and methanolic extracts of the bark of *Acacia nilotica*. It was therefore decided to evaluate these two extracts for antitussive action in mice using two different models that used chemical induction of cough. The results obtained from the study against ammonium liquor induced cough and sulfur dioxide induced cough in mice are represented in Table 3 and 4.

It was obvious from the results that the antitussive action was much more significant in the methanolic extract as compared to the ethyl acetate extract. The dose of 500 mg/kg of the methanolic extract was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models (Figure 1 and 2). The results indicate that the bark extract of *Acacia nilotica* demonstrated antitussive effect in *in vivo* experimental models by prolonging the latency period of coughing and also reducing the frequency of coughing bouts in mice. The increase in latency as well as the decrease in the frequency of coughing bouts occurred in a dose dependent fashion in both the extracts (Figure 3).

Thus, it was observed that oral administration of the extract reduced the frequency of cough in a dose related fashion which was statistically significant when compared with the control group.

CONCLUSION

It can be concluded form the study that as reported in the folkloric literature, the bark extract of plant *Acacia nilotica* has significant anti-cough effect in experimentally induced cough reflex in mice like the standard drug (codeine phosphate). It can be assumed that the extract might be acting via the central nervous system. Further work related to the isolation and characterization of the active constituents as well as evaluation of the mechanism of antitussive effect will be carried out in our laboratory in the near future.

Table 2. Organoleptic Characters of *Acacia nilotica*

Plant parts	Color	Odour	Taste
Flowers	Yellow	Sweet scented	Not evaluated
Leaves	Green colour	Nil	Slightly Bitter
Stem	Brown color	Nil	Slightly Bitter
Bark	Reddish-Brown color	Nil	Not evaluated

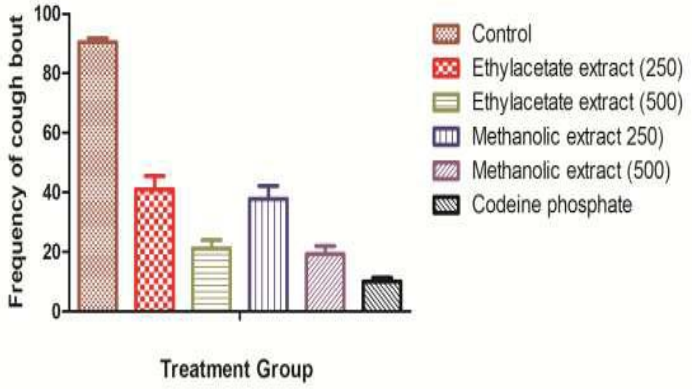

Figure 1. Frequency of Coughing Bouts against Ammonium Liquor Induced Cough.

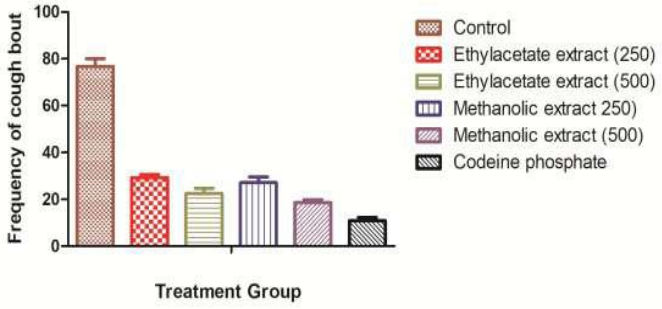

Figure 2. Frequency of coughing bouts against sulfur dioxide induced cough

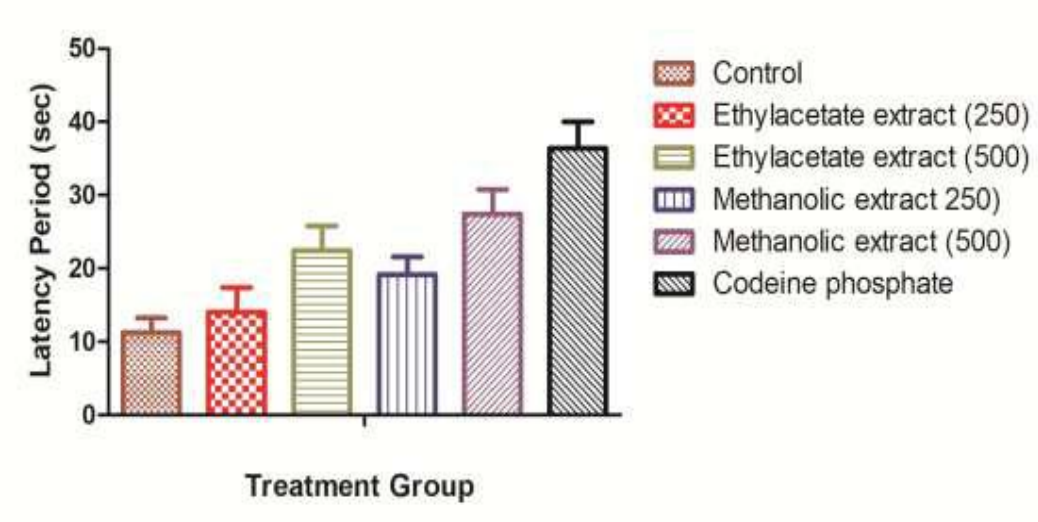

Figure 3. Observed latency period for coughing bouts

Table 3: Antitussive effect of *Acacia nilotica* extract against Ammonium liquor induced coughing

Group	Treatment	Dose (mg/kg, p.o)	Latency Period (Sec)	Number of coughing bouts	Percent Inhibition of cough bout
I	Control	10	11.22 ± 2.01	90.5 ± 1.41	-
II	Ethyl acetate Extract	250	14.02 ± 2.36*	41.06 ±4.36*	54.6
III	Ethyl acetate Extract	500	22.44 ±3.39***	21.24 ±2.71***	76.5
IV	Methanolic extract	250	19.13 ±2.45**	37.79 ±4.35*	58.2
V	Methanolic extract	500	27.44 ± 3.33***	19.33 ±2.62***	78.6
VI	Codeine phosphate	10	36.39 ±3.65***	10.08 ±1.21***	88.9

Values expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, and ***P<0.001 for comparison of treated groups with control

Table 4: Antitussive effect of *Acacia nilotica* extract against sulfur dioxide induced coughing

Group	Treatment	Dose (mg/kg, p.o)	Latency Period (Sec)	Number of coughing bouts	Percent Inhibition of cough bout
I	Control	10	11.22 ± 2.01	76.73 ± 3.32	-
II	Ethyl acetate Extract	250	14.02 ± 2.36*	29.33 ± 1.19*	61.8
III	Ethyl acetate Extract	500	22.44 ±3.39***	22.47 ± 2.21**	70.7
IV	Methanolic extract	250	19.13 ±2.45**	27.14 ± 2.44***	64.6
V	Methanolic extract	500	27.44 ± 3.33***	18.66 ±1.17***	75.7
VI	Codeine phosphate	10	36.39 ±3.65***	10.78±1.44***	86

Values expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, and ***P<0.001 for comparison of treated groups with control

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