ANTITUSSIVE EFFECT OF FRACTIONS ISOLATED FROM METHANOLIC EXTRACT OF ACACIA NILOTICA

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Abstract:

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 a major role in the treatment of human diseases. It can be concluded form the study that as reported in the folkoric literature, the bark extract of plant Acacia nilotica has significant anti-cough effect in experimentally induced cough reflex in mice like the standard drug (codeinephosphate). It can be assumed that the extract might be acting via the central nervous system. 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 F2 fraction as compared to F1 and F3 isolates. The dose of 250 mg/kg of the fraction F2 was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models.

Keywords: Antitussive, Acacia, Cough therapy, AEC inhibitor, Gastro-oesophageal reflux disease.

INTRODUCTION

Now a days 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/semisynthetic 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 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.

Cough

A cough is a symptom of a variety of respiratory and non-respiratory conditions[4] which could be both mild and serious in nature and can be described as "a forced expulsive manoeuvre against a closed glottis that are associated with a characteristic sound or sounds".[5] Causes of cough can range from a common cold to malignancy and pharmacists should be able to distinguish between a cough not resulting from a serious pathology and one which could be the underlying symptom of a potentially critical condition. Studies have shown that the reporting of cough is more prevalent in females than males, possibly due to an increased sensitivity of cough reflex in women.[6]

TYPES OF COUGH

An acute cough is often the result of an upper respiratory tract infection and although distressing it is usually self-limiting and does not require any medical intervention. An acute cough is

defined as one lasting less than three weeks.[7] When a patient presents with an acute cough the pharmacist should still enquire about haemoptysis, the possibility of an inhaled foreign body and prominent systemic illness since cough can be the first indication of an underlying serious condition.[8]

COMMON CAUSES OF COUGH

The following are just a few of the most common causes of cough:

1. ***Angiotensin-Converting-Enzyme (ACE) Inhibitor induced cough:*** One of the most common causes of a dry, non-productive persistent cough, which could be easily detected by a pharmacist, is the one caused by angiotensin converting-enzyme (ACE) inhibitors.[9]
2. **Asthma, COPD, Acute bronchitis, Chronic bronchitis and Eosinophilic bronchitis:** Acute cough is one of the most common symptoms associated with loss of asthma control, acute exacerbations of asthma and COPD. In cough variant asthma, cough may be the only presenting manifestation5 and the treatment is the same as asthma in general.[10]
3. **Environment and smoking induced cough:** Exposure to pollutants and environmental irritants can be a cause of chronic cough. One of the commonest causes of a persistent cough is smoking. One study observed nocturnal cough in relation to indoor exposure to cat allergens. Therefore, patients presenting with cough should be asked about occupational and environmental causes.
4. **Gastro-oesophageal reflux disease (GORD):** Gastro-oesophageal reflux disease, alone or in combination with other conditions, is one of the most common causes of chronic cough. A cough in the absence of gastrointestinal symptoms may be the only presenting complaint in patients suffering from GORD, since there may be no gastrointestinal symptoms up to 75% of the time. It may take two to three months of intensive medical therapy before cough starts to improve and on average five to six months before the cough resolves. Therefore, it is incorrect to assume that cough is not caused by GORD when gastrointestinal symptoms improve or disappear but the cough remains unchanged.[11]
5. **Heart failure:** Acute cough can be the presenting sign of heart failure in patients who have pulmonary congestion. Therefore, since cough can be a symptom of pulmonary

oedema it is important to have a high index of suspicion of left ventricular heart failure in elderly patients presenting with a new or worsening cough, and refer such patients to a specialist.

6. **Malignancy:** Patients presenting with cough and have risk factors for lung cancer should be referred to a specialist. Cough may be due to the cancer itself, the treatment, or other co-existing disease.
7. **Upper respiratory tract infections:** Upper respiratory tract viral infections are one of the most common causes of acute cough, which appears to arise from the stimulation of the cough reflex in the upper respiratory tract by postnasal drip, clearing of the throat, or both.

ANTITUSSIVES

Antitussives are remedies that alleviate coughing. Some antitussives 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.[12] 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, Sitopaladi choorna*, and *Shirisharista* are reported to possess significant antitussive activity.

COLLECTION OF PLANT MATERIAL

Stem bark of the plant under investigation i.e., *Acacia nilitica*was collected from nearby areas of Bhopal (M.P), India in the month of March 2022. The taxonomical identification and authentication of the plant material was done at Safia science college. The specimen of plant samplehas been submitted and preserved in the herbarium of the institute.

MATERIALS

Chemicals

Petroleum ether, Chloroform, Methanol, Ethyl acetate, Glacial acetic acid, Acetone, Formic acid, Benzene, N-propanol, Ethanol, Tween-80, Dimethylsulphoxide (DMSO), Glycerine, conc. Sulphuric acid, Hydrochloric acid, Benzene, N-Butanol, Dichloromethane, n-hexane, pyridine, toluene, xylene, aniisaldehyde, α-napthol, bismuth carbonate, calcium chloride, copper sulphate, Ferric chloride, Follin's reagent, Iodine, Lead acetate, Magnesium chloride, Mercuric chloride, Ninhydrin, Nitric acid, Phloroglucinol, Potassium iodide, Potassium Dichromate, Potassium sodium Tartarate, Ruthenium red, Safranine, Sodium acetate, Sodium iodide, Sodium hydroxide, Sodium nitroprusside. All the chemicals (S.D Fine Chemicals Pvt. Ltd. Mumbai) were purchased from local supplier and were of analytical grade.

Preparation of Acacia nilotica powder

The stem bark of Acacia niloticawas 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.[13]

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.[14]

SUCCESSIVE SOLVENT EXTRACTION

The collected, cleaned powder ofstem 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 h except aqueous, separately.[15] 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.

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/2020/11). 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 h light/10 h 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.[16]

Animal were divided into 5 groups of 6 animals each for each protocol. Group I served as control and was administered with normal saline, group II, III & IV served as treatment groups and were administered with extract of *Acacia nilotica* whereas group V served as positive control and was administered with codeine phosphate, 20 mg/kg p.o (Table 1).

***IN-VIVO*SULPHUR DIOXIDE INDUCED COUGHING**

The *in-vivo* antitussive activity against sulphurdioxide (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 ($NaHSO_3$, 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. Mice were exposed to sulphur dioxide gas as per the experiment model shown in Figure 1 for inductionof cough. Concentrated sulphuricacid (H_2SO_4; CDH, New Delhi, India) was introduced into thevial 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 placedin 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.

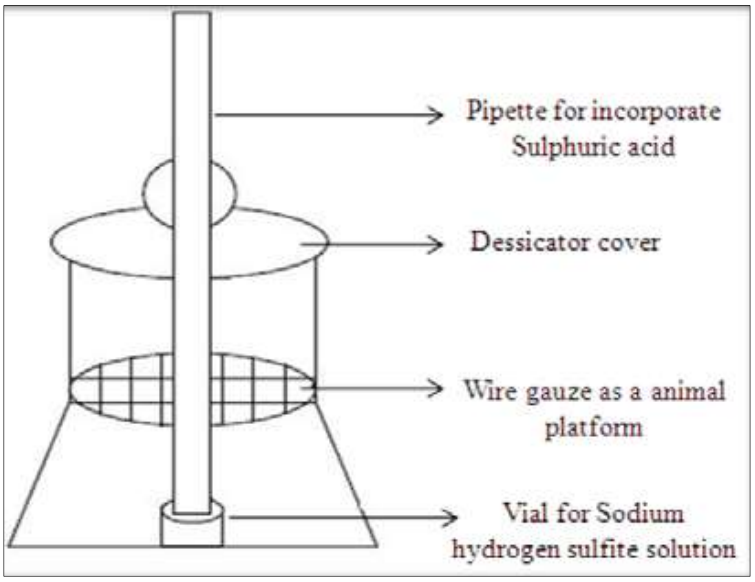

Figure 2 Schematic representation of apparatus used in study

Table 2 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	F1	250	6
Group III	F2	250	6
Group IV	F3	250	6
Group V	Codeine phosphate	20	6

Scoring of Cough Bouts

Before administration of drug, the frequency of cough bout was observed for all the animal groups at 0 min. The frequency of occurrence of cough bout was counted for each animal in each group for a period of 5 minutes after exposure to the stimulus. The animal of the treated groups were exposed to the stimuli chemical 1 hour after administration of the extract.

IN-VIVO AMMONIUM LIQUOR INDUCED COUGH ACTIVITY

The *in-vivo* antitussive activity against ammonium liquor (NH_3) induced cough was evaluated. Healthy mice were selected and divided into seven groups: After 1h 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%) vapors 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.

RESULT AND DISCUSSION

Macroscopic and microscopic evaluation

Organoleptic evaluation represents observing of those properties of materials for whichsense 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 3.

Table 3: 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

It was evident from the macroscopic evaluation of the plant material that the yellow colored flowers have round head and velvety texture, the bipinnate leaves with hairy axis belonged to the plant *Acacia nilotica*. The microscopic features of the bark were observed under a microscope and revealed the presence of thick epidermal cells (Figure 3).

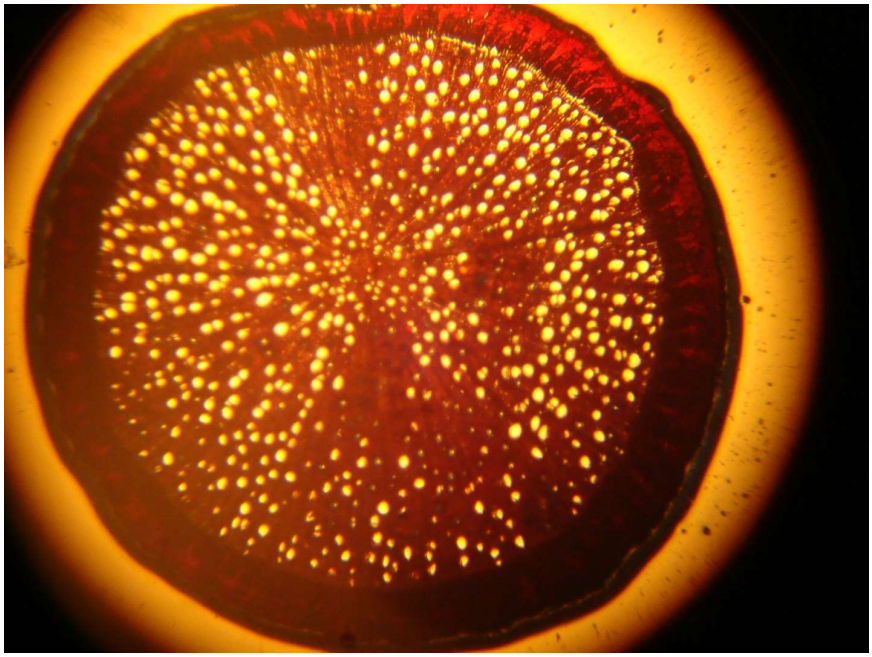

Figure 3: T.S. of *Acacia nilotica* bark exhibiting thick epidermis and xylem cells

The powder microscopy revealed the presence starch granules and xylem vessels in the dried powder of the bark (Figure 4 and 5).

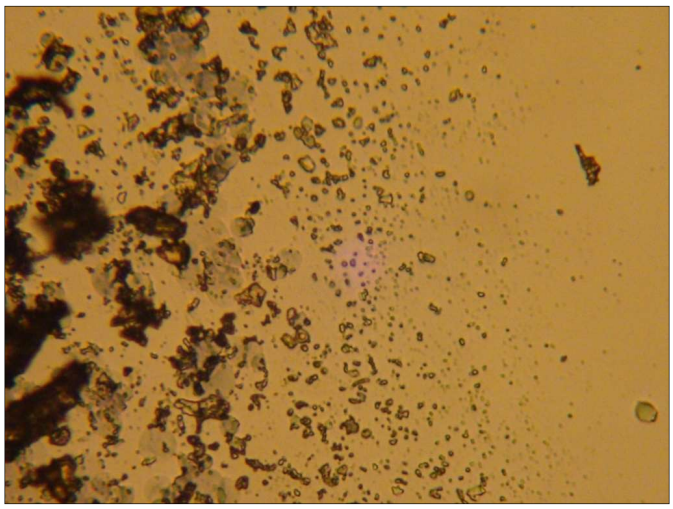

Figure 4: Starch granules visible under light microscope in the bark powder

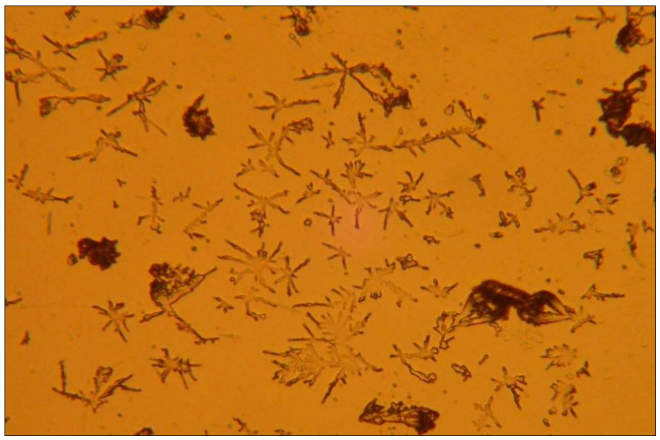

Figure 5: Xylem vessels visible under light microscope in the bark powder

Determination of Percentage yield

The crude extracts so obtained after each of the successive soxhlet extraction process were concentrated on water bath by evaporation of the solvents to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of the bark extracts obtained from different solvents is presented in Table 4.

Table 4: Results of Percentage yield of Bark Extracts

Plant Name	Percentage yield				
	Pet. Ether	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Water**
Acacia nilotica	1.8	1.23	2.03	4.43	6.9

Estimation of Physical Parameters

Determination of ash value

The ash values are useful to determine the quality and purity of the crude drugs. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like calcium oxalates, silica and carbonate content of the crude drug affects the total ash value. Such variables are removed by

treating with acid (as they are soluble in hydrochloric acid). The observed ash values are reported in Table 5.

Table 5: Ash value of plant material

S. No.	Total ash (% w/w)	Acid Insoluble Ash (% w/w)	Water soluble Ash (% w/w)
1	10.2%	6.8%	2.3%

Determination of extractive values

Extractive values of crude drugs are useful for the evaluation of crude drugs. It gives an idea about the nature of the chemical constituents present in crude drug. It is useful for the estimation of specific constituents, soluble in that particular solvent used for the extraction of the drug.

Table 6: Extractive value of plant material

S. No.	Alcohol soluble extractive value (% w/w)	Water soluble extractive value (% w/w)	Ether soluble extractive value (% w/w)
1	15.1%	9.8%	11.1%

Loss on Drying

This parameter is used to determine the amount of moisture present in a particular sample. The amount of moisture affects the powder characteristics as it may cause the growth of microbes. The total moisture content present in the dried bark was found to be 2.35%.

Phytochemical Evaluation

A small portion of the dried extracts were subjected to the phytochemical for presence alkaloids, glycosides, tannins, saponins, flavonoids and terpenoid separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The result of qualitative phytochemical analysisis depicted in the Table 7.

Table 7: Phytochemical analysis of different bark extracts

Chemical Tests	Pet. Ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Aqueous extract
Alkaloids					
Mayer's reagent	-	-	-	-	+
Hager's reagent	-	-	-	-	+
Wagner's reagent	-	-	-	-	+
Dragendorff's reagent	-	-	-	-	+
Glycosides (+Ve)					
Baljet test	-	+	+	+	-
Legal's test	-	-	+	+	-
Keller-Kiliani	-	+	+	-	-
Phenols/Tannins					
Ferric chloride	-	-	+	+	+
Gelatin Solution	-	-	+	+	+
Lead acetate test	-	-	+	+	+
Flavonoids					
$FeCl_3$ test	-	-	+	+	-
Alkaline reagent test	-	-	+	+	-
Shinoda test	-	-	+	+	-
Saponins					
Foam test	-	-	-	-	+

Hemolytic test	-	-	-	-	+
Lead acetate	-	-	-	-	+
Fixed oil/Fats					
Spot	+	-	-	-	-
Saponification	+	-	-	-	-
Gums & Mucilage					
Water	-	-	-	-	-
Carbohydrates					
Molish test	-	-	+	+	-
Fehling's solution test	-	-	+	+	-
Benedict's test	-	-	-	+	-

(+) Indicates 'Presence'; (-) Indicates 'Absence'

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 whereasflavonoids were obtained in the ethyl acetate extract and methanolic extract. Saponins were tested positive only in the aqueous extracts whereas glycosides were detected in the chloroform, ethyl acetate and methanolic extracts. Carbohydrates were found in the ethylacetate and methanolic extracts of the bark.

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 fractions of methanolic 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] X 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 triterpenoidswere present in the ethylacetate and methanolic extracts of the bark of *Acacia nilotica*. It was therefore decided to perform the fractionation of the methanolic extract and evaluate these fractions 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 8 and 9.

Table 8: 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	10.18 ± 2.79	90.5 ± 1.41	-
II	F1	250	13.46 ± 1.36*	44.06 ± 3.36*	51.1
III	F2	250	24.04 ± 3.01***	19.41 ± 1.11***	78.8

IV	F3	250	17.13 ± 1.45**	42.79 ± 3.53*	53.3
V	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 9: 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	F1	250	14.02 ± 2.66*	37.66 ± 1.19*	61.8
III	F2	250	21.02 ± 1.39***	21.07 ± 2.21**	72.3
IV	F3	250	18.71 ± 1.33**	27.14 ± 1.33***	64.4
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

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 was observed.

It was obvious from the results that the antitussive action was much more significant in the F2 fraction as compared to F1 and F3 isolates. The dose of 250 mg/kg of the fraction F2 was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models

Summary

As it was evident from the review of the literature that the bark of *Acacia nilotica* was scientifically the least explored part, in the present work it was therefore envisioned to explore the folkoric reported cough suppressant activity of the bark of the plant. The plant material was collected from the local places of Bhopal in the month of March and was authenticated by macroscopic and microscopic characterization. The macroscopic characters evaluated included the shape, size, taste, smell, texture and color of the different parts of the plant including leaves, stem, bark and flowers. 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 total ash value of the bark was found to be 10.2% while the acid insoluble as was 6.8% and the water soluble ash was found to be 2.3%. The alcohol soluble extractive value was found to be 15.1% w/w, water soluble extractives 9.8% and ether soluble extractive value was found to be 11.1% w/w. Successive solvent extraction of the bark powder was carried out in various solvents including petroleum ether, chloroform, ethyl acetate, methanol and water and the qualitative phytochemical tests were performed on each extract for determination of the presence of alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrates, steroids and other phytochemicals.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. Saponins were tested positive only in the aqueous extracts whereas glycosides were detected in the chloroform, ethyl acetate and methanolic extracts. Carbohydrates were found in the ethylacetate and methanolic extracts of the bark. 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 ethylacetate and methanolic extracts of the bark of *Acacia nilotica*. It was therefore decided to perform the fractionation of the methanolic extract and evaluate these fractions for antitussive action in mice using two different models that used chemical induction of cough. 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 F2 fraction as compared to F1 and F3 isolates. The dose of 250 mg/kg of the fraction F2 was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models.

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