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Research Article

Isolation, Phytochemical and Biological Evaluation of Acacia Nilotica (L) Willd. Leaf Extract.

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ABSTRACT

The present study is to systematically evaluate smooth muscle relaxant activity against Acetylcholine and Oxytocin induced contraction in Wistar rats. Methanolic extract of Acacia nilotica (L) Willd leaf extract were carried out by soxhlet extraction and separation of phytoconstituents by using Thin Layer Chromatography. Identification of phytoconstituents was done by using different physicochemical parameters and smooth muscle relaxant activity was observed against Acetylcholine and Oxytocin induced contraction. The results of the present study concluded that the methanolic extract of Acacia nilotica (L) Willd leaf extract has smooth muscle relaxant activity against both Acetylcholine and Oxytocin induced contraction on duodenum and uterus respectively. Triterpenoid present in the methanolic extract responsible for smooth muscle relaxant activity.

Keywords: Acacia nilotica, Smooth muscle, Triterpenoids,

INTRODUCTION [1, 2, 3]

Acacia nilotica (L) Willd is also known as gum Arabica tree. Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50^0 C) but is frost tender when young.

Plant description:

Acacia nilotica is a single stemmed plant, grows to 15 to 18 m in height and 2-3 m in diameter.

Leaves: The leaves are bipinnate, pinnate 3-10 pairs, 1.3-3.8 cm long, leaflets 10-20 pairs and 2-5 mm long. The leaves of the plant have shown already Chemo protective, Anti-mutagenic, Antibacterial, Astringent, Antimicrobial, Antiulcer, Anti-inflammatory activity. The leaves have potential smooth muscle relaxant activity which is tried to find in this study.

MATERIALS AND METHODS

Plant material: The fresh leaves of Acacia nilotica Linn. Willd were collected in the month of August 2012 from the local area of Sangli region, Maharashtra. The plant was authenticated. The 950 gm of plant leaves were collected then sorted carefully and washed thoroughly to remove dirt and debris. The plant material was spread out in thin layers on drying trays, kept in shade for 15 days and turned frequently. Thus leaves were dried naturally. The dried material was weighed and found to be 350 gm.

Extraction: [4] In the present study, the plant leaves was carefully selected and shade dried. The dried leaves was reduced to powder in the mechanical grinder and passed through a sieve no.40 to obtain powder of desired size. About 350 gm of powdered material was kept for soaking in Petroleum ether (60-80) for 48 hr to remove fatty material from plant leaves powder. Then defatted material is subjected to exhaustive extraction with methanol in a soxhlet extractor at a temperature of 30^0c.

350 gm of powdered drug gives 33.2 gm of extract

Therefore, 100 gm of powdered drug gives x gm of extract.

$$X = \frac{100 \times 33.2}{350}$$

X = 9.48%

Percent practical yield= 9.48%

Phytochemical screening: Identification of phytoconstituents is done by performing following tests.

Separation and isolation: [5, 6] The methanol extract was subjected to thin layer chromatography. Different solvent systems as mobile phase were tried to obtain the proper resolution and the mobile phase giving clear and distinct spot was selected for further work. Constituents were separated on thin-layer chromatographic plates in chloroform: Methanol (8:2) solvent system. The bands observered on the preparative plates were isolated by scrubbing and then by adding it in to ether and subjected to centrifugation. Finally the compound remained after evaporation will be stored in proper storage condition for further use of it. The best separation of three distinct spots was obtained with the mobile phase Chloroform: Methanol (8:2).

Pharmacological screening: [7, 8] Female Wistar rats 200-250 gm was housed in animal house of the institution maintained under standard conditions and were handled with ethical guidelines. Rats were starved but not banned from water for 24 hr before experiment.

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Table no. 1: Extractive value determination

Extract	Color	Wt. of drug taken (gm)	Wt. of product obtained (gm)	% practical yield
Methanol	Bluish green	350	33.2	9.48

Table no. 2: Phytochemical investigation of methanolic extract of Acacia nilotica

Sr. no.	Chemical test	Results
1.	Test for Sterol's	
	Salkowaski test	+
	Liebermann Burchard test	+
2.	Test for glycosides	
	Test for cardiac glycosides	+
	Test for Anthraquinone glycoside's	–
3.	Test for Saponins	–
4.	Test for Carbohydrate's	+
5.	Test for Flavonoid's	+
6.	Test for Alkaloid's	+
7.	Test for tannin's	+

Table no. 3: Identification properties

Criterion	Property recorded
Physical properties	
Melting point	215^0 C
Solubility	Chloroform, DCM, Methanol.
Colour	Brownish.
Colour on TLC plate after spraying Anisaldehyde	Violet

Table no. 4: Dose response of Acetylcholine on isolated rat duodenum.

Dose	Height of response	% Relaxation	Response
Ach (0.1 ml)	2.9	-	Contraction
Ach (0.2 ml)	3.2	-	Contraction
Ach (0.4 ml)	3.8	-	Contraction
Ach (0.8 ml)	2.9	-	Contraction

Table no. 5: Effect of methanolic extract of Acacia nilotica (MEAN) on Acetylcholine induced contraction on isolated rat duodenum.

Dose	Height of response	% Relaxation	Response
Ach (0.4 ml) + MEAN (0.0 ml)	3.8	-	Contraction
Ach (0.4 ml) + MEAN (0.1 ml)	3.1	18.43	Relaxation
Ach (0.4 ml) + MEAN (0.2 ml)	2.1	44.74	Relaxation
Ach (0.4 ml) + MEAN (0.3 ml)	1.5	60.53	Relaxation
Ach (0.4 ml) + MEAN (0.4 ml)	0.7	81.58	Relaxation

Procedure: After discarding the 10 cm nearest to the gastro duodenal junction, the duodenum muscle strips (15 to 20 mm), free from adhering tissues, were removed from Wistar rats and set up for recording the isotonic contractions in 5 ml jacketed organ baths containing Tyrode solution at 37^0 C, continuously bubbled with air under 1 gm of load. After an equilibrium period of at least 30- 45 minutes, cumulative concentration curves for acetylcholine (10nM to 100 µM), were obtained for the absence or presence of different concentrations of Methanolic extract (0.1-3.0 mg/ml), and incubated for 15 minutes. The maximum response obtained from the first cumulative concentration-effect curve was taken as the 100% response value. Same experiment performed on Rat uterus in which Oxytocin was used to induce contraction and physiological salt solution used was De-Jalone solution.

Smooth muscle relaxant activity: For this activity, methanolic extract of leaves of *Acacia nilotica (L)* Willd was used. Bioassay was carried out by using Sherrington's rotating drum machine for screening the smooth muscle relaxant activity of methanolic extract. Acetylcholine and Oxytocin were used to induce contraction on isolated rat duodenum and uterus respectively.

Rat duodenum: The dose response curve relationship of Acetylcholine where height of response increases with dose and sealing effect was observed at 0.4 ml. In order to observe inhibitory effect the sealing effect dose of 0.4 ml is kept constant and dose of MEAN (Methanolic Extract of Acacia Nilotica) is increased. The results showed that as dose of MEAN increases, the inhibition increases.

Rat uterus: The dose response curve relationship of Oxytocin where height of response increases with dose and sealing effect was observed at 0.4 ml. In order to observe

Table no. 6: Dose response of Oxytocin on isolated rat uterus.

Dose	Height of response	% Relaxation	Response
Oxytocin (0.1 ml)	4.1	-	Contraction
Oxytocin(0.2 ml)	6.6	-	Contraction
Oxytocin (0.4 ml)	8.0	-	Contraction
Oxytocin (0.8 ml)	5.4	-	Contraction

inhibitory effect the sealing effect dose of 0.4 ml is kept constant and dose of MEAN is increased. The results showed that as dose of MEAN increases, the inhibition increases.

Table no. 7: Effect of methanolic extract of Acacia nilotica (MEAN) on Oxytocin induced contraction on isolated rat uterus.

Dose	Height of response	% Relaxation	Response
Oxy. (0.4 ml) + MEAN (0.0 ml)	8.0	-	Contraction
Oxy. (0.4 ml) + MEAN (0.1 ml)	6.6	17.5	Relaxation
Oxy. (0.4 ml) + MEAN (0.2 ml)	5.7	28.75	Relaxation
Oxy. (0.4 ml) + MEAN (0.3 ml)	4.2	47.5	Relaxation
Oxy. (0.4 ml) + MEAN (0.4 ml)	2.7	66.25	Relaxation

IR spectra obtained from A.B.C.P. Sangli.

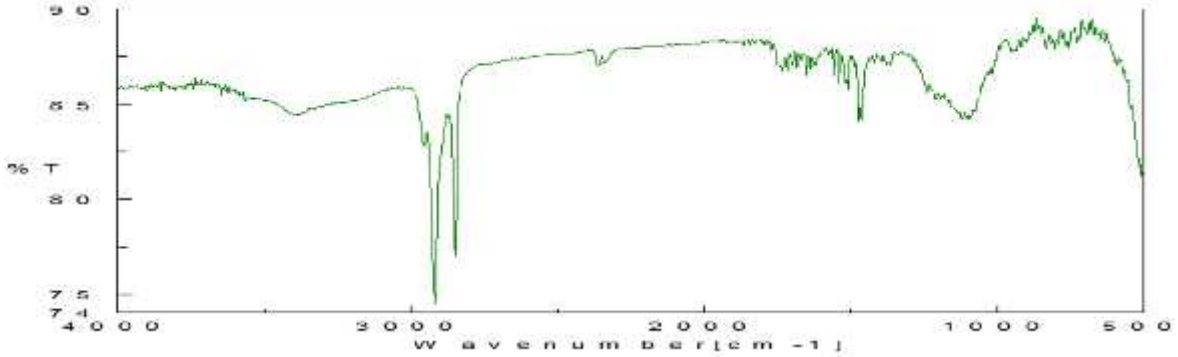

Fig. 1: IR spectrum of isolated compound (S3)

^{1}H NMR Spectra was obtained from IIT, Powai, Mumbai.

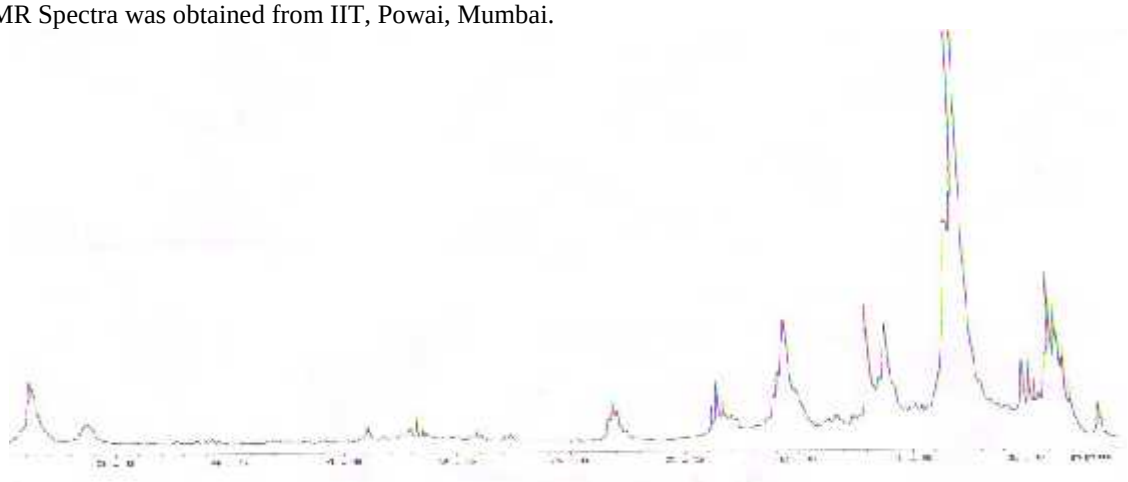

Fig. 2: ^{1}H NMR analysis of isolated phytoconstituent (S3)

Identification of compound: [9, 10]

Structure elucidation of isolated compound (S3):

Structural elucidation of isolated compound by IR: A characteristic absorption band appeared at 3383.5 cm^{-1} and was assigned to the stretching vibrations of the Hydroxyl group while another absorption band at 2956.34 cm^{-1} was intensified and assigned to the stretching vibration of the methylene group. Furthermore absorption appeared in the region of 1652.7 cm^{-1} is characteristic of alkenes group and characteristic absorption appeared at 833.09 cm^{-1} suggesting the existence of aromatic ring. All these absorption peaks reveals that the compound may be Lupeol a triterpenoid.

Structural elucidation of isolated compound by NMR: Isolated compound S3 is a pentacyclic triterpenes. ^{1}H NMR spectrum of S3 showed seven tertiary methyl singlets at δ 0.85, 0.88, 0.89, 0.95, 0.97, 1.08 and 1.25. Its chemical shift at δ 3.66 represents hydroxyl group. It also showed Olefinic proton at δ 5.35 and 5.36.

Table no. 7: Effect of methanolic extract of Acacia nilotica (MEAN) on Oxytocin induced contraction on isolated rat uterus.

Dose	Height of response	% Relaxation	Response
Oxy. (0.4 ml) + MEAN (0.0 ml)	8.0	-	Contraction
Oxy. (0.4 ml) + MEAN (0.1 ml)	6.6	17.5	Relaxation
Oxy. (0.4 ml) + MEAN (0.2 ml)	5.7	28.75	Relaxation
Oxy. (0.4 ml) + MEAN (0.3 ml)	4.2	47.5	Relaxation
Oxy. (0.4 ml) + MEAN (0.4 ml)	2.7	66.25	Relaxation

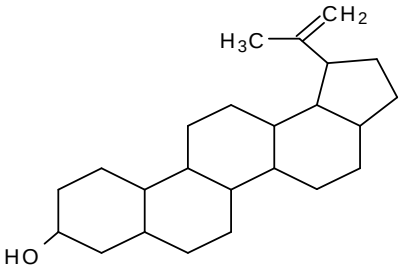

Fig. 6: Lupeol

RESULT AND DISCUSSION

From the above observation table it was found that methanolic extract of leaves of Acacia nilotica (L) Willd. showed excellent smooth muscle relaxant activity.

Acetylcholine is known to open receptor operated calcium channels and releases calcium from its storage sites, thus inducing phasic and tonic contraction. Another mechanism for Acetylcholine induced contractions involves activation of non-selective cation channels in the plasma membrane, which results in membrane depolarization. The depolarization stimulates Ca^{2+} influx through voltage-gated Ca^{2+} channels.

Action of Oxytocin in myometrium is independent of innervations. There are specific G- protein coupled Oxytocin receptors which mediates the response mainly by depolarization of muscle fibers and influx of ca^{2+} ions as well as through phosphoinositide hydrolysis and IP_3 mediates intracellular release of ca^{2+} ions. The number of Oxytocin receptor increases markedly during later part of pregnancy. Oxytocin increases PG synthesis and release by endometrium which may contribute to the contractile response. Distinct subtypes of Oxytocin receptors have been shown on the myometrium and the endometrium.

CONCLUSION

From chromatographical data (TLC), preliminary qualitative analysis, spectroscopic analysis (^{1}H NMR, IR) and melting point suggested that the isolated compound was Lupeol a triterpenoid.

Pharmacological screening of methanolic extract of leaves of Acacia nilotica (L) Willd. showed better results for smooth muscle relaxation activity.

Thus, exploration of the chemical compounds of plants will provide us the basis for developing a lead molecule in pharmaceutical industry.

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