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Phytochemical screening and analgesic activity of methanolic extract of *Ximenia americana*.

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Abstract
The analgesic activity of the methanol extract of *Ximenia americana* leaf extract (MEXA) was investigated and studied by narcotic models like hot plate and tail flick tests. MEXA at doses of 200,400 and 600mg /kg i.p and the standard drug (morphine 1.5mg/kg body weight, i.p) the duration of reaction time (sec) increased dose dependently and significantly compared to the control group. In the tail flick test, the plant extract produced dose dependent increase in reaction time which was significantly higher in the test and standard group compared to the control group. The plant possesses significant antinociceptive property as evidenced in all the animal models of nociception. It might possibly exert its effect through diverse mechanism that may involve both central and peripheral pathways. The preliminary phytochemical investigation revealed the presence of tannins, saponins, alkaloids, glycosides, flavonoids, triterpinoids, sterols and phenolic compounds in the methanolic extract of leaves of *X. americana* which may be responsible for its antinociceptive activity.

Key Words
Xeminia americana, Hot plate, nociception, Tail flick test.

Introduction
Ximenia americana is a small tree or shrub which grows widely in the topical and temperature reasons in the world and belongs to family Olacaceae. It is commonly known as false sandal wood, number if plants, which are known to posses medicines properties, have been in use in the folklore medicine. According to tribal literature available, the *Ximenia americana* leaves and twigs are used in treating fever and cold. The roots are used in skin problems, headache, sleeping sickness, edema, dysentery, mouth ulcers and as a antidote in poison. In northern parts of Nigeria this plant is used for treating Malaria[1].The fruits are used in habitual medicine and in diabetic[2].The aqueous plant material extract is also used in the treatment of cancer in African Traditional medicine. The oral evidence indicator that the plant is effective in these conditions but there is no documented scientific evidence to support such use. Hence, the present work was under taken to evaluate the analgesic property and phytochemical screening of methanolic extract of leaves of *Ximenia americana*.

MATERIALS AND METHODS

Plant material
The leaves *Ximenia americana* collected from the Nallamala forest of Chintala village, Dornalamandal, Ongole District, Andhra Pradesh in the month of September 2010 and were taxonomically identified by Botanist of S.V.University and a specimen voucher NO.1295 is kept for future reference at S.V.University.

Preparation of extract
The collected leaves were shade dried coarsely powdered by using mechanical grinder and stored in airtight container. The powder was extracted with methanol using soxhlet apparatus. The solvent was then removed under reduced pressure .It was further concentrated and dried in the desiccators for further studies. The percentage yield of the extract is 13%.

Phytochemical Screening
A preliminary phytochemical screening of methanol extract was carried out as described by Khandelwal K.L[3].

Chemicals
Morphine sulphate was purchased from sigma (Poole, UK), Methanol from merck Limited, Mumbai – 400018.

Animals
Healthy adult swiss albino mice of either sex, approximately of same age, weighing between 25-30g and adult male Sprague Dawley rats weighing between 180-200 g were used for the study. They were housed under controlled conditions at $25^0 \pm 3^0$ C, 50 ± 5% RH and kept under 10/14 h light / dark

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cycles with food and water adlibitum. Animals were group housed in polypropylene cages containing sterile paddy husk bedding. The study was conducted after obtaining the approval of the Institutional animal ethics committee. The animals were fasted for 14 h before test to achieve better drug absorption through gastro intestinal tract.

Dosing schedule

The animals were randomly allocated into 5 groups of 6 each. Group I was assigned as control, group II, III and IV received *Ximenia americana* (MEXA) 200,400 & 600 mg/kg ip[4] body weight, respectively and group V received the standard drug, i.e morphine sulphate 1.5 mg/kg body weight, intraperitoneally in hot plate and tail flick tests.

Screening for analgesic activity by Eddy's hot plate

In the hot plate test[5],mice of either sex were placed on the hot plate (Rolex) maintained at $55^0 \pm 0.5^0$c .The time between placement on the hot plate and the occurrence of licking of the paws ,shaking or jumping off from the plate was recorded as response latency .Mice with basal latency of more than 10 sec were not included in the study .The response latencies or reaction time was measured before administration (basal) and at 30 and 60 min after administration of the test compound (200 , 400 & 600mg/kg i.p) or the standard drug (morphine sulphate 1.5 mg/kg ,i.p) and compared with the control group . A cut-off reaction time was fixed at 20 sec to avoid damage to the paws.

Screening of analgesic activity by tail flick method

In this model, Nichrome wire analgesiometer (Rolex) was used[6].Individually , the tail of each rat was placed over the radiant heat source of the apparatus and the tail withdrawal from the heat (flicking response) was taken as the end point .The reaction time (sec) of each rat in each group was determined at 0,30 and 60 min following administration of the test compound (200, 400 & 600 mg/kg i.p) or the standard drug , morphine sulphate (1.5 mg/kg , i.p) and compared with the control .

Statistical analysis

The results were subjected to statistical analysis as per standard statistical analysis as per standard statistical method[7].

Results

Phytochemical screening of the methanolic extract of Xeminia Americana revealed the presence of tannins, saponins, alkaloids, glycosides, flavonoids, triterpenoids, sterols and phenolic compounds. In the hot plate test, there was significant ($p<0.01$) increase in reaction time in *X.americana* (200-600 mg/kg) as well as morphine sulphate (1.5mg/kg) treated group, from 0 to 60 min (Table 1). However, in the control group the duration of reaction time did not increase upto the end of the study period. Reaction time at 60 min of observation *X.americana* [200 (7.142sec), 400 (9.517sec) and 900mg/kg (10.103sec)] as well as morphine sulphate 1.5mg/kg (15.568sec) treated groups was significantly ($p<0.01$) higher compared to the control group (6.895sec) indicating dose and time dependant anti nociceptive activity of the test plant. However, the standard drug showed better analgesic activities than the plant extract. In the tail flick test, the reaction time (sec) increased significantly ($p<0.01$) from 30 to 60 min after single peritoneal administration of *X.americana* (200,400&600mg/kg) and morphine sulphate (1.5mg/kg, i.p) from 3.54 to 5.122, 3.18 to 9.17 and 3.31 to 16.2sec, respectively. However, with 300mg/kg of X.americana and the control group, there was no significant increase in the reaction time from 30 to 60 min of observation period (Table 1) indicating dose and time dependant analgesic activity of the plant.

Discussion

In the present study, anti nociceptive effect of methanolic extract of the leaves of *Ximenia americana* was evaluated in different experimental models of pain i.e, narcotic models like hot plate and tail flick tests. The results of the present study clearly demonstrated that the methanolic extract of *Ximenia americana* possessed a definite dose dependant anti nociceptive activity as observed by significant increase in the reaction time in hot plate and tail flick test as compared to the control group. The hot plate and tail flick are the most common tests of nociception that are based on a phasic stimulus of high intensity. The nociceptive experience is short lasting and it is well accepted that agonists of μ – opoid receptors produce analgesia in acute pain models[8].

Therefore, it is believed that substances that are effective in tail flick exert their effects predominantly through μ – opoid receptors. The hot plate test is considered to be selective for opioid-like compounds, which are centrally acting analgesics in several animal species[9].The methanolic extract of

X.americana had antinociceptive activity in hot plate test that may in part be mediated by opioid receptors .These findings indicate that the methanolic extract of X.americana may extort sufficiently opioid like compounds out of the plant which are responsible for the analgesic activity of the plant. Leukotriene as well as peptidoleukotrienes result in an increase in vascular permeability and chemotaxis of polymorphonuclear leucocytes, which sensitize nociceptors[10,11]. As the plant under study also contain triterpene as one of its phytoconstituents, so it may act through inhibition of leukotriene biosynthesis. The presence of alkaloid in the plant extract supports the claim that this compound have antinociceptive property since, alkaloid, flavonoids and saponins have been found in other natural products with analgesic and anti – inflammatory properties[(12,13)]. It may also be related partly to the presence of steroids that have been shown to exert analgesic effects in animal models of nociception[14]. The plant extract exhibited antinociceptive activity in all the animal models of nociception and possibly exerted its effect through diverse mechanism that may involve both central and peripheral pathways. Further pharmacodynamics investigations are required to understand the precise mechanism of antinociception exhibited by the methanolic extract of leaves of X.americana.

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Table 1: Antinociceptive activity of X.americana in hot plate and tail flick test.

Group	Dose mg/kg	Hot Plate Test			Tail Flick Test		
		Reaction Time (SEC)			Reaction Time (SEC)		
		0 min	30 min	60 min	0 min	30 min	60 min
I (control)	10ml/kg	6.56 ±0.42	6.72 ±0.42	6.90 ±0.28	5.23 ±0.23	5.57 ±0.87	5.88 ±0.40
II (X.americana)	200mg/kg	6.04 ±0.65	6.96 ±0.45	7.14 ±0.78	5.47 ±0.47	5.60 ±0.36	5.80 ±0.39
III	400mg/kg	5.87 ±0.78	7.21 ±0.74	9.52 ±0.60	3.54 ±0.25	4.53 ±0.29	5.12 ±0.54
IV	600mg/kg	6.17 ±0.63	8.85 ±0.71	10.10 ±0.84	3.18 ±0.28	7.14 ±0.43	9.17 ±0.64
V (standard)	1.5mg/kg	6.86 ±0.34	11.90 ±0.37	15.57 ±0.32	3.31 ±0.27	6.10 ±0.34	16.20 ±0.63

Values are mean ± SE of 6 animals, P<0.01.