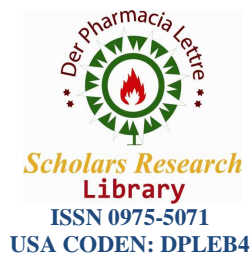




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Anti-inflammatory and analgesic study of fibrous part of *Adansonia digitata* fruit using microwave extraction techniques

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ABSTRACT

Adansoniadigitata is a majestic tree revered in Africa for its medicinal and nutritional value. The plant parts are used to various diseases such as diarrhoea, malaria and microbial infections. It is reported that biobab is an excellent anti-oxidant due to the vitamin C content which is seven to ten times higher than the vitamin C content of oranges. Baobab has numerous biological properties including antimicrobial, antiviral, anti-oxidant and anti-inflammatory activities amongst others. Phytochemical investigation revealed the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals. The seeds are a source of significant quantities of lysine, thiamine, calcium and iron. Baobab is an important commodity which is integral to the livelihood of rural communities. In addition, the global demand for baobab raw material (e.g. seed oil, fruit pulp) by the food and beverage, nutraceutical and cosmetic industries has increased dramatically in recent years thereby increasing the commercial value and importance of this coveted African tree. In the past few years, there has been an increased demand for non-timber forest products (NTFPs), specifically baobab seed oil for inclusion in cosmetic formulations due to its high fatty acid composition. This review summarises the botanical aspects, extraction of plant ash product using different solvent, biological properties of extracted crudes.

Keywords: Adansonia Digitata, Baobab, Analgesic Activity, Anti-inflammatory Activity.

INTRODUCTION

Baobab is a large and well known African tree (Fig. 1) belongs to family Bombacaceae.^[1] The baobab has been referred as “arbre a palabre”, meaning the place in the village where the elders meet to resolve problems.^[2] Literature reveals that many parts of the plant especially leaves, fruits and seeds, fibres have been used in medicinal purposes.^[3] Chemical constituents of the plant have been mentioned in several reports, however many reports reveal the composition and indicate the characters of seed oil.^[4] The plant has excellent nutritive values, leaves, seeds and composition of the leaves have been investigated.^[5] Glucosides and Flavanonol have been reported in the root bark.^[6] The medicinal application includes treatments for skin, problems of intestine and several uses as Analgesic, Anti-inflammatory, Anti-pyretic agents.^[7]

Inflammation is a very common and often associated with many diseases and can occur in many organs and tissue. Moreover the acute inflammatory action is a normal part of our immune response to infection and injury. To address the additional applications of the plant to the treatment in inflammatory condition as well as for the bacterial

treatment, the fruit extract was collected and was evaluated for its Analgesic, anti-inflammatory and Anti-bacterial properties.

In this study we prepared the extract in Toulene, Dichloromathane, Pet-ether, Methanol, water and DMSO using Microwave and supercritical fluid techniques.

1. Botanical Aspects

1.1. Name History of *A. digitata*

The origin of Baobab name is uncertain, however scientists believe it is derived from the Arabic name buhibab meaning fruit endowed many seeds. The genus name *Adansonia* is used in honour of Michel Adanson (1727–1806) who brought seeds to Paris in 1754 and who was the first person to provide a comprehensive description accompanied by a drawing of the plant after a trip to West Africa (Senegal).^[8] The species name *digitata* (hand-like) was selected in reference to the shape of the leaves. Several names are used to describe the baobab depending on its geographical location and include “magic tree”, “chemist tree”, “symbol of the earth”, “upside-down tree” and “monkey bread of Africa” amongst numerous others.^[9]

1.2. Botanical background and habitat

Total eight baobab species have been identified around the globe and six species found on the island of Madagascar are endemic to that region.^[10] It is postulated that the centre of evolutionary origin of the genus *Adansonia* is Madagascar.^[11] The African species *A. digitata* is indigenous to, and widely distributed throughout the savannas and savannah woodlands of sub-Saharan Africa. The only species which is not endemic to the African continent is *A. gibbosa* (A.Cunn.) Guym. ex A.Baum native to Australia. In southern Africa, *A. digitata* is commonly found in Malawi, Zimbabwe, Mozambique and South Africa especially in the warm parts of the Limpopo Province, while in West Africa, it is found in Mali, Benin, Senegal, the Ivory Coast, Cameroon and Burkina Faso. In East Africa, the plant is found in countries such as Kenya, Uganda and Tanzania.^[12] The baobab is a massive deciduous tree easily distinguishable by its huge trunk. It is regarded as the largest succulent in USA markets and the demand for these products are increasing. An increased demand can lead to overexploitation of the plant therefore it is important to determine the factors that could lead to the successful cultivation of this commercially important tree. Ecological niche modelling studies were undertaken to determine factors that are crucial to the cultivation of baobab and results indicated that annual precipitation and seasonal temperature fluctuations were two key factors.

2.3 Medicinal uses of *A. digitata*

Various parts of the plant are used to treat almost all the diseases. However the documentation endowed with the treatment of diarrhoea, anaemia, dysentery, toothache and body ache.^[12] Indian people use pulp with buttermilk for control of diarrhoea, dysentery and young leaves for inflammation purposes.^[13] Moreover the dried leaves are used in South Africa for mosquito repellent purposes;^[14] The pulp of dried fruit is reported for control of arthritis disease.^[15] In South Africa and rest of world the parts of the plants are used for nutritional purposes.

The plant is extremely useful for human in dry area of Africa since it offers raw materials for many useful items. In West Africa tree trunk is used for water storage for longer time.^[16]

MATERIALS AND METHODS

3.1 Plant material

Plant sampling was carried out during the growing season of (January–June 2014) from Dr Babasaheb Ambedkar Marathwada University campus, Aurangabad (Maharashtra state). At the time of collection, two pressed herbarium specimens were prepared and identified with the help of Plant taxonomist; Department of Botany, Maulana Azad College, Azad campus, Aurangabad.

3.2 Extraction method

3.2.1 Microwave Extraction

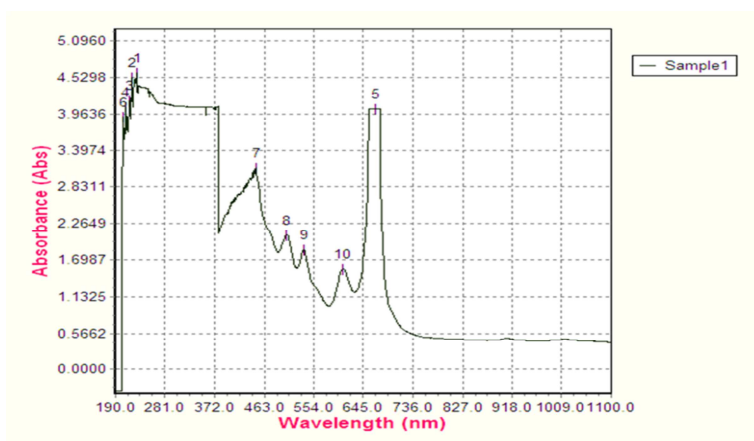
The dried external fibrous part of fruit was burned in open air. Black powder of ash was mixed thoroughly and then extracted sequentially with toluene, dichloromethane, Hexane, methanol, DMSO, MeOH: H₂O (1:1) and water (100 - 1000 ml, Ranchem and Qualigens), using microwave (Catalyst System.7, Model Cata R i). The solvent from the extract was evaporated under reduced pressure of 22–26 mm Hg at 45–50°C by ‘Rotavapour’ and the residue obtained was stored at 0–5°C in an amber vial under nitrogen atmosphere.



The extractive values are mentioned in Table No 1

3.3 UV-Visible Spectra of Extracted crude

UV-visible spectral analysis of *Adasoniadigitata* in methanolic extract shows 10 peaks and absorbance at 650 nm.



2. Biological activities

4.1 In Vivo Anti-inflammatory Activity

Carrageenan induced rat hind paw edema method^[17, 18]. Healthy Swiss albino rats weighing 150-190g were used in this experiment. Required number of albino rats of either sex was divided into required number of groups and they were numbered individually. Animals were fasted 24 hours before administration of drugs with water ad libitum. Animals were marked on their hind paw just beyond tibiotarsal junction to ensure constant dipping in the mercury column up to a fixed mark. Initial paw volume (both right and left) of each rat was noted by mercury displacement method using plethysmograph. Group I was marked as a control which was administered with 0.1 ml of 1% carrageenan solution. Group II received ibuprofen at a dose of 10.6 mg/200 g body weight along with carrageenan which was served as standard. Group III to last number received test samples along with the inflammogen. After the drug treatment, 0.1 ml of 1% w/v carrageenan solution was injected in to plantar region of the left paw of the standard and test groups. The paw volumes of both the legs of control and standard groups was measured with the help of plethysmograph for 30 min, 1 h, 2 hrs and 4 hrs after carrageenan administration. The percentages of inhibition of inflammation in the drug treated animals were recorded and calculated using the formula

$$\% \text{ inhibition Volume} = (W_t - W_c) / W_t$$

Wherein,

W_t: Mean oedema volume control

W_c: Mean edema volume of drug.

Ibuprofen was used as standard drug in aqueous suspension prepared with 0.2% w/v solution of CMC as suspending agent. Samples of synthesized compound were prepared as aqueous suspension with 0.2% w/v solution of CMC as suspending agent.

The results are summarized in Table No 2.

4.2 Analgesic activity

Acetic acid induced writhing method was adopted for evaluation of analgesic activity^[19]. Healthy Swiss albino mice were used for these experiments. Required numbers of healthy albino rats weighing 20-25g were used in the experiments. After 30 min of administration of drugs writhing was induced by intraperitoneal injection of 1% acetic acid in volume of 0.1 ml/10g body weight and writhing episodes were recorded for 15 minutes.

The percent protection against the writhing episodes were recorded and calculated by using formula

$$\% \text{ protection} = (1 - W_t/W_c) \times 100$$

Where in,

W_t = Mean of the writhing episodes in the test.

W_c = Mean of the writhing episodes in control.

Aspirin dispersed in water (dose – 100mg/Kg) was used as a standard Drug. Synthesized compounds were prepared as an aqueous suspension using 0.2% CMC as suspending agent and administered orally. (Dose –100mg/Kg body weight.)

The results are summarized in Table No 3.

RESULTS AND DISCUSSION

5.1 Anti-inflammatory Activity

The evaluations of anti-inflammatory activity of naturally extracted crude were performed by the carrageenan-induced rat paw edema method using ibuprofen as reference drug. Mean changes in paw oedema thickness after 30 min, 1 hr, 2 hrs. and 4 hrs. from induction of inflammation and inhibition % of oedema by the by the tested compounds were recorded in Table No 2.

The extracted material exhibited excellent anti-inflammatory activity. Total seven samples were isolated using different solvents for extraction. Methanolic extract showed maximum Anti-inflammatory profile. Combination of water and MeOH for extraction showed poor biological profile value when compared with methanol as such. The activity profile observed to be good when polar solvent DMSO used for extraction purpose. The extraction collected using non polar solvent hexane shown poor % extraction along with poor activity profiles in Anti-inflammatory segments. Also the activity profiles for solvents toluene and dichloromethane was not as good as MeOH, moreover the % extraction values for dichloromethane and toluene were also less than 0.5%.

4.2 Analgesic activity

The isolated natural crude extract from *A. Digitata* was evaluated for their Analgesic profile using Aspirin as a standard reference drug. Methanolic extract displayed maximum Analgesic profile. The extract collected using polar solvent DMSO also exhibited excellent profile; the extract collected using 1:1 methanol and water showed decreased in profile compare to methanol. Moreover the Analgesic activity profile of extract collected from hexane, dichloromethane and toluene was poor along with extraction % values.

Table 1 The % Extraction Values of Adansonia Digitata

Extract	Powder Quantity (g)	Solvent Amount (ml)	Crude Qunantity	% Extraction of extract (g)
MeOH	100	1000	1.85	1.85
DMSO	100	100	0.85	0.85
H ₂ O	100	1000	1.60	1.60
MeOH:H ₂ O	100	1000	1.38	1.38
Toulene	100	1000	0.43	< 0.5
Dichloromethane	100	1000	0.48	< 0.5
Hexane	100	1000	0.22	< 0.5

Table 2 The anti-inflammatory activities of isolated crude Extraxt of A. Digitata

Extract	Std Error Mean	% Inhibition	p-Value
MeOH	0.02	64.21	< 0.01
DMSO	0.03	46.21	< 0.01
H2O	0.01	38.00	< 0.01
MeOH:H2O	0.04	62.12	< 0.02
Toulene	0.02	12.12	< 0.01
Dichloromethane	0.03	23.46	< 0.03
Hexane	0.01	3.01	< 0.01
Control	0.04	00	----
Ibuprofen	0.04	91.6	< 0.01

Table 3 Analgesic Activities of crude Extrat of A,Digitata

S.No.	Std Error Mean	% Inhibition	Writhing Episode	p-Value
MeOH	0.87	55.31	10.33	Non-Significant
DMSO	1.11	51.26	16	Non-Significant
H2O	1.82	43.9	912	Non-Significant
MeOH:H2O	1.17	42.28	16	Non-Significant
Toluene	1.12	27.00	12	Non-Significant
Dichloromethane	1.14	29.89	9.0	Non-Significant
Hexane	1.12	8.81	7.0	Non-Significant
Control	0.55	---	21.66	-----
Aspirin	1.05	60	8.66	< 0.01



Fig 1
A-Fruit of Adansonia Digitata
B-Adansonia Digitata Tree
C-Fruit and Seed

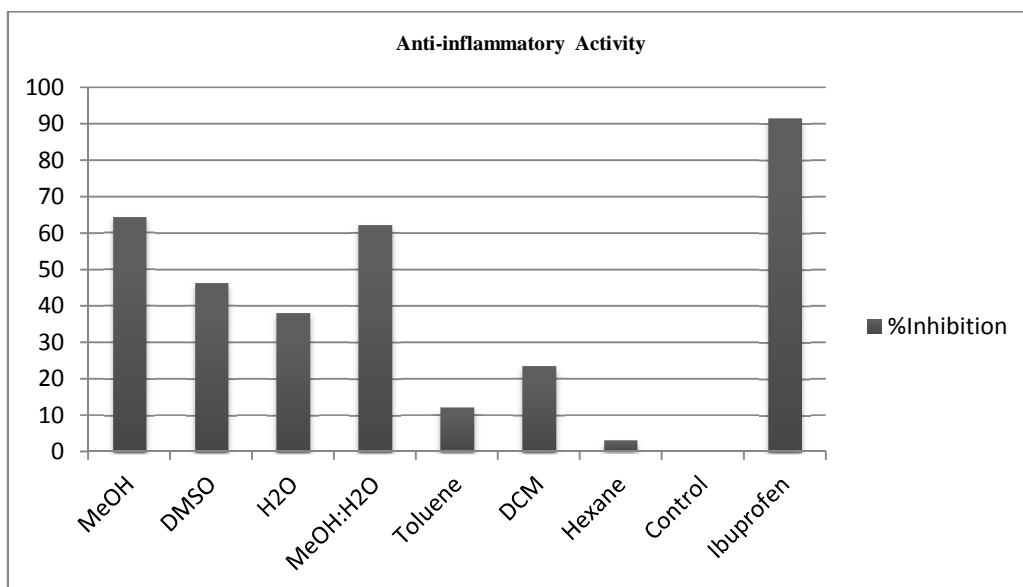


Fig 2 Graphical Representation of Anti-inflammatory Activities

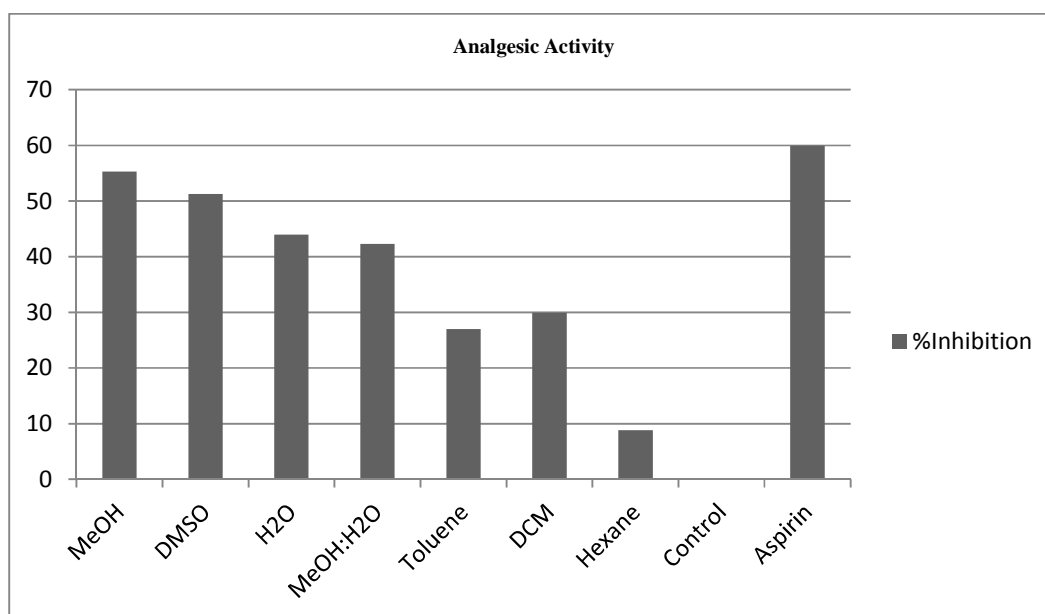


Fig 3 Graphical Representation of Analgesic activities

CONCLUSION

The present article deals with the extraction and biological evaluations of fibrous material of fruits part of *Adenсонia Digitata* plant, extracted using different solvents. Summarizing the biological results, the mentioned biological activity clearly reveals that the tested extracts of methanol and DMSO showed promising Analgesic and Anti-inflammatory profile. In Analgesic era the extract was found almost equipotent with standard reference Aspirin.

The results reported in this study open the possibility for further investigational developments in Anti-inflammatory and Analgesic segments for the fibrous part of *A. digitata* fruit.

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