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Experimental Validation of Ethnic Knowledge of Abortifacient Plant *Abutilon persicum* (Burm.F.) Merr Used by Tribes of Idukki, Kerala

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ANJU MOL M.R, PARVEEN NISHA*, KUMUTHAKALAVALLI.R, AJESH.T.P

DEPRTMENT OF BIOLOGY, GANDHIGRAM RURAL INSTITUTE-DEEMED UNIVERSITY, GANDHIGRAM-624 302

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ABSTRACT

To determine the abortifacient potential of *Abutilon persicum* (Burm.F.) Merr using ethno gynaecological knowledge. The methanol extraction of entire plant was subjected to phytochemical analysis (standard methods) and the major phytocompounds such as alkaloids, carbohydrates, flavonoids, tannins, phenols, terpenoids, glycosides, and saponins were confirmed. The antioxidant activities (DPPH assay) of the plant extract was ranging from 19.6±0.04 to 71.4±0.54% with the half effective concentration was (EC50) value of 77µg/ml. The antifertility phytoconstituents (saponins) were separated using solvent fractionation (water and butanol 1:1) of *A.persicum* were Di-N octyl phthalate and methylene chloride that are similar to the synthetic abortifacient compounds namely trichloroethylene and hepta chloride which was identified through GC-MS analysis. The scientific evaluations prove the ethnic knowledge of *Abutilon persicum* as an abortifacient.

INTRODUCTION

The traditional knowledge of tribals is critical to Science and Society for managing natural resources for sustaining human health, for ensuring economic stability and for improving the quality of life. Ethnobotany is the study of such knowledge system which gives scope to know much more about the plant-human relationship, practical applications, and utilization of tribal knowledge to medicines of various pharmacological functions. In recent times bio-prospecting of biodiversity is being undertaken for identifying viable plants as the possible source of more effective and marketable drugs. In this context, ethnobotanical information is very useful and promising. The present study deals with the scientific evaluation of an abortifacient plant namely *Abutilon persicum* (Burm.f.) Merry, an ethnic information not validated so far.

EXPERIMENTAL

Using the primary data of ethnogynoecological studies of the tribes in Idukki District, Kerala, India (1) the whole plant of *Abutilon persicum* was collected, authenticated and the voucher specimen was deposited in the herbarium repository of the Institute.

Preparation of the plant extract

The fresh plant material was thoroughly washed with tap water, shade dried, powdered and stored in airtight containers.

Extractive value

20gm of powdered plant material was then extracted using 200ml of methanol in soxhlet apparatus for about 24hrs and the extract was stored at 4^0C to carry out further experiments.

Phytochemical analysis

The Qualitative phytochemical analysis of alkaloids, phenols, flavonoids, tannins, terpenoids, saponins, glycosides and polysaccharides of the plant extract were evaluated (2).

Antioxidant assay

0.1mM solution of DPPH was prepared in 100% methanol, and 1 ml of this solution was added to 4 ml of sample in 40% methanol at various concentrations (10-100µl). This mixture

was shaken vigorously and incubated for 30 min at 37^0C in the dark. The violet color was changed to pale yellow color. The reduction of the DPPH radical was measured by continuous monitoring of the decrease in absorption at 517 nm using ELISA reader having L-ascorbic acid as standard (3).

DPPH scavenging effect (%) = (A0-A1/A0) ×100

Where A0 was the absorbance of the control reaction and A1 was the absorbance recorded in the sample.

Phytoconstituents responsible for antifertility

Saponins are reported to have a relationship with sex hormones (4). Hence in this study, the plant sample was subjected to separation of saponins and GC-MS analysis.

Solvent fractionation and saponin separation

40gm of the dried plant powder of *Abutilon persicum* was successively extracted with 60 % of petroleum ether for 24hrs; re-extracted with methanol for 24 hrs and concentrated using rotary evaporator.

From the crude extract, the saponins were separated using solvent fractionation of butanol and water (1:1ratio) using Silica gel Column Chromatography. The eluted sample was subjected to GC-MS assay hyphenated to high-resolution mass spectrometer (5)

GC-MS analysis

GC-MS analysis of the saponin fractions of *A.persicum* was performed using a Clarus 680 Perkin Elmer gas chromatography equipped with an Elite-5 capillary column (5% diphenyl, 95% dimethyl polysiloxane) (30.0m × 0.25mmID × 250m) and mass detector turbo mass of the company which was operated in EI mode. Helium was the carrier gas used at a flow rate of 1mL/min. The injector was operated at 200^0C and the oven temperature was programmed as follows: 60^0C for 2min and 10^0C/min until 300^0C. Interpretation of GC-MS was carried out using the database of National Institute Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained (6).

RESULTS

Characteristics of *Abutilon persicum*

The plant *Abutilon persicum* (Burm.f) Merr. belongs to the family Malvaceae. The plant is commonly known as "Persian malo". It is a dicot, erect pubescent undershrub grows up to 1.2m height. This plant is often used as a medicinal plant and is considered invasive on certain tropical Islands. The plant is distributed in districts like Wayanad, Palakkad, Idukki of Kerala state; Coimbatore, Dindigul Salem, Tiruchirapalli, and Nilgris of Tamilnadu state. The plant species occur in a number of tropical Sub- tropical Islands. In Tamil Nadu, it is by local name called as 'Thuthi'. It is also known as by various vernacular names like velvetleaf, China jute, buttonweed. It grows up to 1-2 m height. The aerial part of the plant *A. persicum* is used in folk medicine as expectorant and emollient. Various species of *Abutilon* are traditionally claimed for their varied pharmacological and medicinal activity, different parts of the plant contain specific phyto constituent responsible for these activities. The stem yields a long and silky fiber which can be used to make rope (7)

Extractive value

Analysis of phytoconstituents in plant samples will be very much useful in the standardization of crude drugs. The chosen plant sample yielded extractive value of 2.7gm when 20gm of powdered plant material was extracted using 200ml of methanol.

Phytochemical investigation

The phytochemical screening of *A.persicum*, indicated the presence of various important secondary metabolites namely alkaloids, carbohydrates, flavonoids, phenols, terpenoids glycosides and saponins and found to be rich in flavonoids, terpenoids, and saponins (Table 1).

Table.1 Phytochemical screening of plant extract of *Abutilon persicum*

Sr. No.	TESTS	OBSERVATION
1	Test for alkaloids (Mayer's test)	+
2	Test for carbohydrate (Carbohydrate test)	+
3	Test for Flavonoids (Ferric chloride test)	++
4	Test for tannins (Ferric chloride test)	_
5	Test for phenols (Alkaline reagent test)	+
6	Test for terpenoids (Salkowski test)	++
7	Test for glycosides (Borntrager test)	+
8	Test for saponins (foam test)	++

Antioxidant assay

The scavenging activity of the methanolic extract of the plant *Abutilon persicum* was compared with L-Ascorbic acid as standard. The DPPH radical scavenging activity was increased at sample concentration 20 to 100µg/ml. The dose-dependent of DPPH radical scavenging activity of methanolic extract of *A. persicum* was ranging 19.6±0.04 to 71.4±0.54 and the EC_{50} value was recorded as 77µg/ml concentration (Figure 1).

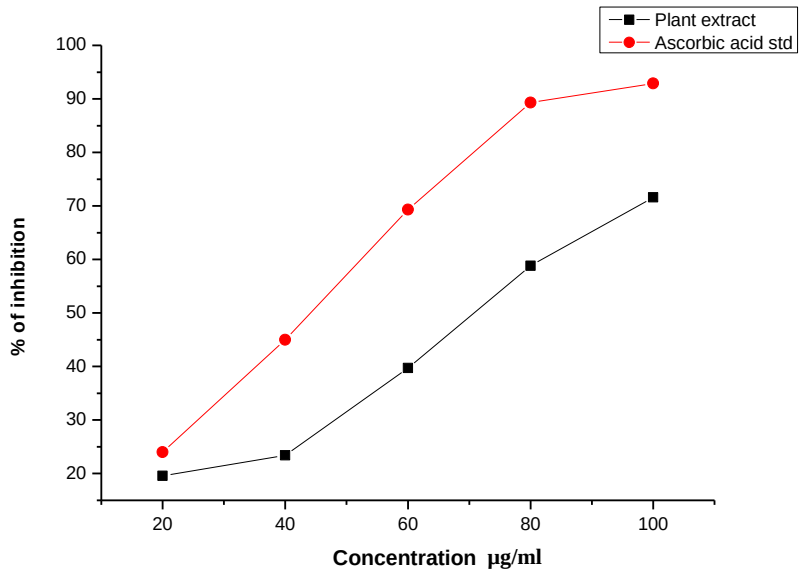


Figure 1 DPPH Radical scavenging activity of *A. persicum*

GC-MS Chromatogram of the plant extract of *Abutilon persicum*

Gas chromatography-mass spectrometry (GC-MS) is a reliable tool for the identification of phytoconstituents based on the peak area, molecular weight, and molecular formula. In this study, seventeen compounds were identified with the help of standard library search software, HPCHEM and the analysis recorded the presence of methylene chloride and Di-n octyl phthalate. The chromatogram contains D_1 –N octyl phthalate ($C_{24}H_{38}O_4$) with the retention time of 23.667 and peak area of 54.493% and methyl chloride (CH_2Cl_2) with the retention time of 2.523 and peak area of 45.507%.

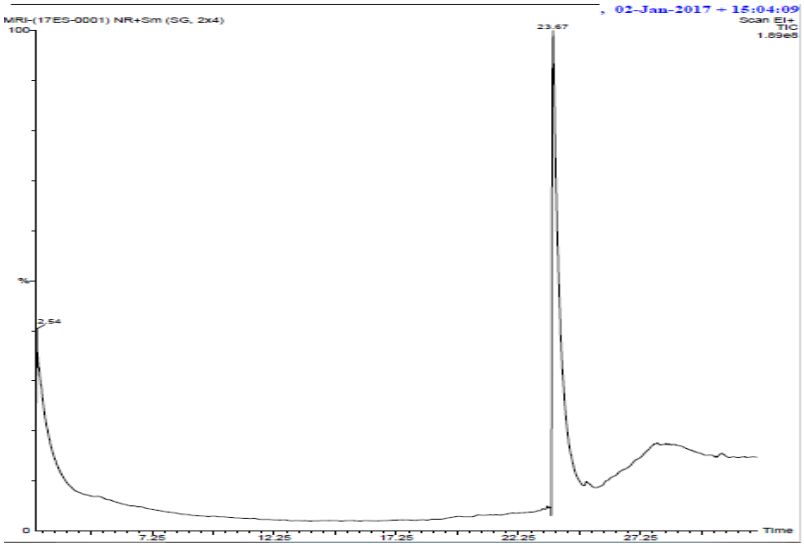
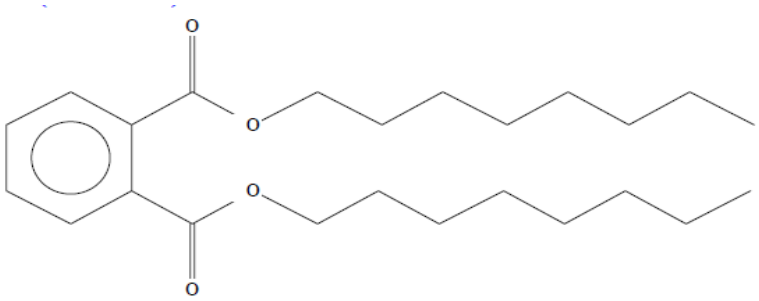


Figure 2: GC-MS Chromatogram of the plant extract of *Abutilon persicum*

Di-N octyl phthalate (a)



Methylene chloride (b)

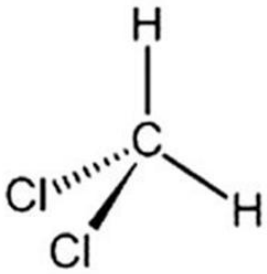


Fig 3: Structure of the compounds Di-N octyl phthalate (a), Methylene chloride (b)

DISCUSSION

The traditional knowledge generated out of the co-evolution and coexistence of both the indigenous cultures and the traditional practices gives scope for bioprospecting of biodiversity. There are some valuable regional records of indigenous plants that are used by tribes to treat infertility, conception and abortion, obstetrics, gynecological disorders and infectious diseases. Some important ethnogynaecological works were published by researchers in different parts of the world (8,9). Medicinal and aromatic plants contribute a major source of natural organic compounds widely used in medicine.

In India, the use of plants as abortifacient is in practice from olden times. Till today tribal populations depend mainly on plants for family planning and to induce abortion (10) Around 29 plants traditionally used by Urali tribal women for various gynaecological purposes like abortion, anaemia, antifertility, to prevent lactation, to clean uterus after delivery and ethnogynoecological observations among Mannan tribes recorded around 47 plants that are used as abortifacient, antifertility drugs and contraceptives among various tribes of Idukki including *Abutilon persicum* (11).

Members of plants belonging to family Malvaceae such as *Abelmoschus esculentus*, *A.manihot* and *Hibiscus rosa sinensis* and *Abutilon persicum* have been reported to be used by tribal women to avoid pregnancy (12, 13). Reports state that tender fruit of *Abelmoschus esculentus*, *Abelmoschus Manihot* is being consumed by the tribal woman to avoid pregnancy (14). Leaves of *Spondia mombin* were reported to be associated with the oxytocic and abortifacient activity (15). Immunomodulatory effect of *Sida cordifolia* (16); the abortifacient effect of *Abutilon persicum* plant juice and honey mixture (17) and the usage of flowers *Hibiscus rosa sinensis* for antifertility and contraception; and usage of few species from

allied genera of Sida such as Abutilon, Pavonia, Urena etc, that are used as 'Bala' one of the medicaments used in the treatment of antitumor, anti HIV, hepatoprotective, abortifacient, immunostimulant properties have been reported (18).

Vascular plants contain the enormous variety of chemical compounds namely secondary metabolites that are the main curative principles in plants. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants still play a vital role to cover the basic health needs in the developing countries. The phytochemical analysis of tribal medicinal plants will help in determining the action of medicine. The 120 active compounds currently isolated from medicinal plants in our medical system; about 74 percent show a positive correlation between their modern therapeutic use and their traditional use (19).

The medical value of these plants lies in their chemically active substances with a variety of structural arrangements and properties (20) that produce a definite physiological action on the human body. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, glycosides and phenolic compounds (21)

In recent times there has been a renewed interest in finding out naturally occurring antioxidants for their applications as pharmaceuticals. Phytochemicals like carotenoids, tocopherols, ascorbates, and phenols are strong antioxidants which play a vital role in a healthcare system. Antioxidants function through defense mechanisms within cells of the human body by inhibiting the formation of free radical species, intercepting radical chain reactions, converting existing free radicals into less harmful molecules and repairing oxidative damage. Antioxidant and free radical scavenging properties are reported in *Sida rhomboidea* (22) Saponins are natural glycosides that exhibit abortifacient activity. The negative effects of saponin on animal reproduction have been long known and have been ascribed to their abortifacient, anti-zygotic property (23). The contents of these phytochemicals may be associated with reported oxytoxic and abortifacient activity of the plant *Abutilon Indicum* leaf extract (24). Some plants contain steroidal saponins from leaves and stems which are important and interest in pharmacy due to their relationship with such compounds as sex hormones (25). Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity as the emergency contraceptive (26). Saponins of *Sesbania sesban* L. were reported to be used as the emergency contraceptive (27).

The presence of di-n-octyl phthalate in *A.persicum* reported in the present study may have the etiological association with endometriosis, with the symptom of abdominal pain, heavy periods and infertility; it may found to have the synergetic effect with trichloroethylene and hepta chloride, in causing parental loss of fetus (28). The ethnic information may have scientific confirmation for the reason that the diethyl hexyl phthalate observed in *Abutilon persicum* may found to have synergic effect with trichloroethylene and hepta chloride, in causing parental loss of fetus as reported earlier (29). So also phthalates may act as an estrogen and are a potential risk factor for estrogen-related diseases such as endometriosis, maternal exposure to diethyl hexyl phthalate lead to adverse delivery outcomes (30). (31) reported that the phthalates can negatively affect reproductive functions in laboratory animals as estrogen and cause estrogen-related diseases such as endometriosis

CONCLUSION

The outcome of the experimental results reveals that the ethno gynoecological knowledge on *A.pesicum* as an abortifacient is a valid information. The presence of saponins with phthalates may be the factors responsible for inducing abortion as phthalic esters are reported to have abortifacient activity and are being used in synthetic medicines to induce abortion. Further investigations and *in-vivo* studies are needed to prove the pharmacological action of the chosen plant.

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