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EVALUATION OF DIURETIC ACTIVITY OF DIOSPYROS MALABARICA PLANT EXTRACTS

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ABSTRACT

Medicinal herbs are the significant source as Diuretics. Mono and poly herbal preparations have been used as diuretics According to one estimate more than 650 mono and poly-herbal preparations in the form of decoction tincture tablets and capsules from more than 75 plants are in clinical use There exist a large number of studies which supports the diuretic effects of traditional herbal medicines This article reviews the various herbal plants used traditionally as diuretics and the identification of chemical constituent of the plant promoting diuresis The present paper also involves various plant drugs and their pharmacological profile which focus on the dose administered bioactive extract involved in diuresis mechanism Presant study focuses on preparing ethanolic fractions of petrolium ether extracts of the diospyros malabarica and evaluation of the prepared ethanolic fractions for the diuretic activity in rats

Key words : Diospyros malabarica, ethanolic fractions, diuretic activity etc.,.

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INTRODUCTION

"Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant The Indian Traditional Medicine like Ayurvedic Siddha and Unani are predominantly based on the use of plant materials Herbal drugs have gained importance and popularity in recent years because of their safety efficacy and cost effectiveness The association of medical plants with other plants in their habitat also influences their medicinal values in some cases One of the important and well -documented uses of plant -products is their use as Diuretic agents Diuretic are commonly defined as drugs that increase the amount of urine output by the kidneys These agents augment the renal excretion of sodium and either chloride or bicarbonate primarily and water excretion secondarily"

MECHANISM OF ACTION OF DIURETICS

"Diuretics play an important role in the management of oedema and hypertension This function is mainly an increase in net negative water and solute balance The proximal convoluted tubule reabsorbs about 50-66 % of fluid by both active and passive processes The thin descending limb of Loop of Henle allows osmotic water abstraction as it is highly permeable to water and impermeable to solutes The reduced water absorption from the descending limb of Loop of Henle has an important role in over-all enhanced condition of diuresis The thin ascending limb of Loop of Henle is impermeable to water and highly permeable to chloride and sodium therefore diuretics show no effects on it"

HERBAL TREATMENT

Medicinal herbs are the significant source of Diuretics Mono and poly-herbal preparations have been used as diuretics According to one estimate more than 650 mono and poly-herbal preparations in the form of decoction tincture tablets and capsules from more than 75 plants are in clinical use

HERB USED AS A DIURETIC

Herb used as a diuretic has been used in India for a long time and has been popularized world over by leading pharmaceuticals Plant medicine was commonly used for traditional treatment of some renal diseases and a lot of

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plants have been reported to show significant diuretic activity Many investigators have demonstrated that studies of herbal plant used in traditional medicine as diuretics have increased recent years and might be a useful tool in the treatment of hypertension Hypertension is considered one of the main and dangerouscomplications of diabetes mellitus

A medicinal plant is any plant which in one or more of its organs contains substances that can be used for therapeutic purposes, which are precursors for chemo- pharmaceutical semi-synthesis When a plant is designated as medicinal it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify

them as articles of drugs and therapeutic agents and are used for medicinal purposes The WHO has estimated that up to 80 % of the population in Africa and the majority of the populations in Asia and Latin America still use TM for their primary healthcare needs Plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine vincristine taxol podophyllotoxin camptothecin cases the crude extract of medicinal plants may be used as medicaments About 121 major plant drugs have been identified for which no synthetic one is currently available Plant parts are usedin all branches of medicine such as Allopathy Homeopathy Unani and the Ayurvedic system The genus *Diospyros malabarica* Ebenaceae which is distributed throughout the tropics is characterized by its ability to produce triterpenes of the lupine series The genus *Diospyros* consists of *ca* 240 species 59 of which are distributed in India Thailand Japan Nigeria South Africa and Philippines *Diospyros malabarica* family Ebenaceae grows throughout India and other tropical regions of the world The main uses of different part of plants are

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used for furniture and wood carvings also used as raw material for boats and constructions Wood density at air-drying is 0.80-1.10 gram/cm, it is very durable and strong and the ripe fruits are edible

The traditional uses of *D. malabarica* are prolong onset of diarrhea reduction of gastrointestinal motility and inhibition of the synthesis of prostaglandin observed The above effects of it may also be due to the presence of gallic acid and like tannins and polyphenols in the extract The protection provided by medicinal plants against oxidative damage to body tissues has been attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants and other bioactive compounds Moreover there is a scope that these plants can be used as a therapeutic agent against various liver diseases as they may possess hepatoprotective activity by virtue of its antioxidative potential Therefore it is a very important the main active ingredients which can be extracted from plants Moreover to clarify their role in the treatment of present diseases, and how they can be used to produce or synthesis more effective drugs

METERIALS & METHODS

Collection and Authentification of Plant Material

The whole plant of diospyros malabarica will be collected and will be authenticated by dr k madhava chetty department of botany sri venkateswara university tirupathy

Extraction of Plant Material

The plant is grinded in to a coarse powder with the help of suitable grinder

Counter-current Extraction

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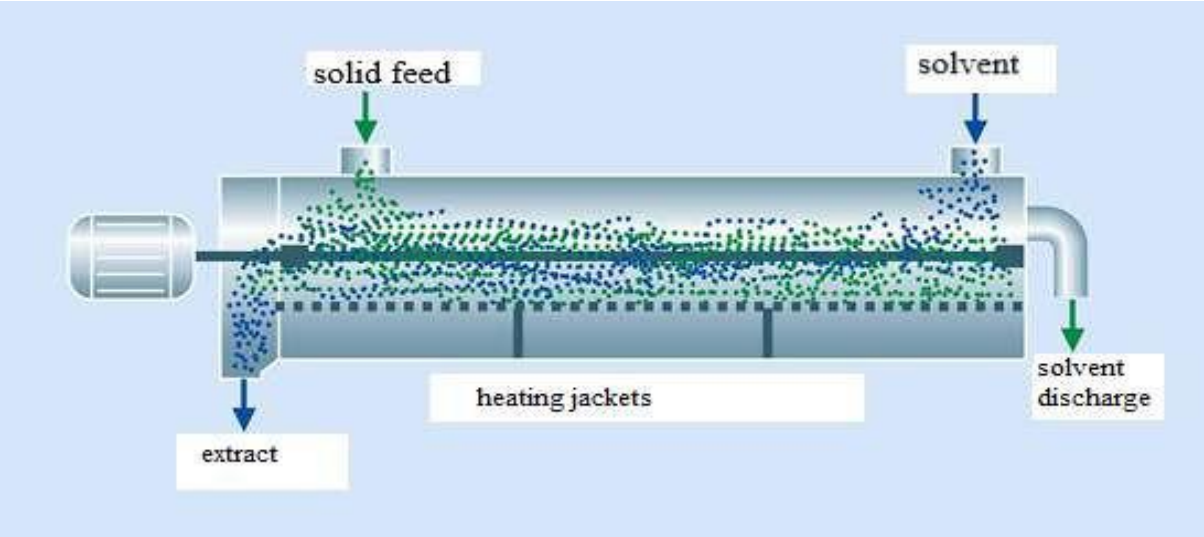
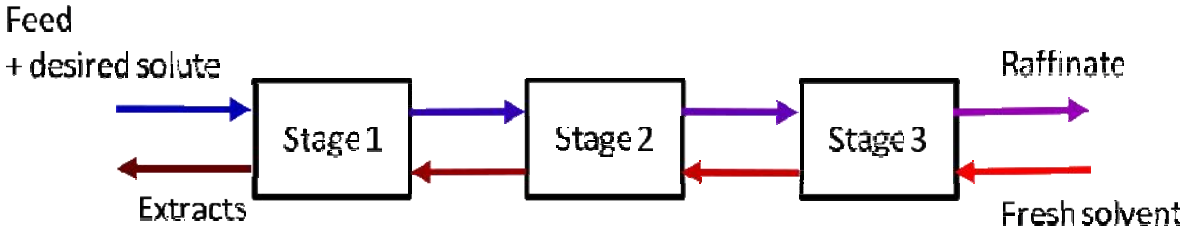
In counter-current extraction (CCE) wet raw material is pulverized using toothed disc disintegrators to produce a fine slurry In this process, the material to be extracted is moved in one direction generally in the form of a fine slurry within a cylindrical extractor where it comes in contact with extraction solvent The further the starting material moves the more concentrated the extract becomes Complete extraction is thus possible when the quantities of solvent and material and their flow rates are optimized The process is highly efficient requiring little time and posing no risk from high temperature Finally sufficiently concentrated extract comes out at one end of the extractor while the marc practically free of visible solvent falls out from the other end





Preparation of ethanolic fractions :

Prepared petrolium ether extracts are taken and mixed with ethanol and mixed thoroughly and kept aside for settling down of the compounds and ethanol phase is separated from the mixture

Evaporation of Solvent The filtrates obtained were evaporated using Rotary evaporator in a porcelain dish They rendered a gummy concentrate of greenish black The extract was kept in vaccum dissecator for 7 days

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Preliminary phytochemical screening

Freshly prepared seed extracts of both plants were tested for the presence of phytochemical constituents by using reported methods Khandelwal K.R 2005 Kokate C.K 1994 Farnsworth N.R, 1966 Evans W.C, 2005.

Animals

Albino rats weighing between 140 and 200 g of either sex were used in the study and were obtained from the sanzyme bio labs pvt. Ltd. hyderabad The experimental protocol was approved by the Institutional Animal Ethical Committee and these animals were used to evaluate the diuretic activity of diospyros malabarica The animals were maintained under standard husbandry conditions for an acclimatization period of 15 days before performing the experiments All rats were housed in metallic cages six in each and temperature maintained at 22±2°C.

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Acute toxicity studies

The study was carried out by limit test in accordance with OECD 423 guideline Albino rats weighing between 140 and 200 g of either sex were divided into two groups control group that received normal saline and a test group that received a limit dose of 5000 mg/kg of the extract orally The animals were deprived of food for 18 hours with free access to water Immediately after administration the animals were carefully observed continuously for the first 4 hour for any overt signs of toxicity and death and then for the next 24 hours Thereafter they were kept under close observation up to 14 days to monitor the presence of any signs of morbidity or mortality The weight of each animal was recorded at the 1st, 7th, and 14th day of administration to verify any weight change that might have occurred Finally after cervical dislocation the rats were dissected at the 14th day to observe gross pathology of the vital organs such as liver and kidney

Experimental Animal Protocol

Diuretic activity (Lipschitz test) Healthy adult Wister rats of either sex weighing 140-200 g procured from sazyme bio labs pvt Ltd. Hyderabad were used for the study The animals were maintained in well ventilated room temperature with natural 12 + 12 day – night cycle in the polypropylene cages The animals were fed with balanced diet that is standard rodent pellet diet Hindustan Lever Ltd and water ad libitum The animals were housed for 1 week prior to the experiment to acclimatize to the laboratory conditions

Wistar rats were divided into six groups (3 each) The animals of

group (I) served as normal control Vehicle which received normal saline water (2 ml/kg b.w., orally) only

The animals of group (II) served as standard control which received Urea (1 g/kg b.w., orally)

Groups (III) to (IV) received extracts respectively at dose of x mg/kg b.w., orally

The method is based on water and sodium excretion in test animals as compared to rats treated with high dose of urea The method of Lipschitz et al was employed for the assessment of diuretic activity

Male Wistar rats weighing 140 to 200 g were used They were placed in metabolic cages provided with a wire mesh bottom and a funnel for collecting the urine Stainless steel sieves

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were placed in the funnel to retain the feces allowing only urine to flow down for collection and measurement The food and water are withdrawn 15 h prior to the test Three animals were placed in one metabolic cage The rats of each group were treated with drugs as per the details

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mentioned above Additionally 5 ml of normal saline solution per 100 g was administered orally to all rats Urine excretion was recorded after 5 h The sodium and potassium contents of the collected urine were estimated by Flame Photometer (Toshniwal group model TCM-35 The instrument was calibrated with standard solutions containing different concentrations of Na+ and K+ The conductivity was directly determined on fresh urine samples using a conductometer Toshniwal group model TCM-15 pH was measured with a digital pH meter MK-VI Unique instruments & machineries Calcutta on fresh urine sample

EFDM (Ethanolic Extract of *Diospyros malabarica*.).

Values are expressed as the mean ± SEM $*p < 0.001$ compared to the control group

$**p < 0.001$ compared to Urea group (ANOVA followed by Dunnett's test) Diuretic index = volume of test group/volume of control group

Effect on urinary electrolyte excretion

Table 2. Effect of oral administration of the ethanolic extract of *Diospyros malabarica* on urinary electrolyte excretion

Treatment	Dose	Total Na^+ (µmoles/kg)	Total K^+ (µmoles/kg)	Total Cl^- (µmoles/kg)	Na^+/K^+ ratio
Normal saline	25 ml/kg	1555 ± 14.02	704 ± 7.34	579 ± 15.01	2.20
Urea	1 gm/kg	3345 ± 4.80**	1345 ± 11.06**	2034 ± 15.78**	2.48
EFDM	25 mg/kg	2043 ± 4.42*	1000 ± 10.11*	2003 ± 10.30*	2.04
EFDM	50 mg/kg	2427 ± 0.73*	1104 ± 6.23*	2095 ± 13.20*	2.19

EFDM (Ethanolic Extract of *Diospyros malabarica*.).

Values are expressed as the mean ± SEM

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*$p < 0.001$ compared to the control group **$p < 0.001$ compared to Urea group ANOVA followed by Dunnett's test

†Na^+/K^+ ratio = Total Na^+/Total K^+.

CONCLUSION

The present study demonstrates the diuretic activity of the ethanolic fractions of Diospyros malabarica which increased urinary volume and electrolyte sodium potassium and chloride excretion The diuretic pattern of the ethanolic fractions was similar to that of the reference drug urea suggesting a similar mechanism of action Further study of Diospyros malabarica is necessary in order to isolate the compounds present in this species as well as identify which compounds are responsible for the diuretic effect shown by the ethanolic fractions Additionally it is necessary to determine the mechanism or mechanisms of action involved in the diuretic effect

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