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RESEARCH ARTICLE

Fruit Extract of *Mallotus philippinensis* Used as a Natural Indicator in Acidimetry Titration

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Manuscript Info *Abstract*

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Mallotus philippinensis species are known to contain different natural compounds, mainly phenols, diterpeniods, steroids, flavonoids, cardenolides, triterpenoids,coumarinans, isocoumarins and many more which exhibit interesting biological activities such as antimicrobial, antioxidant, antiviral, or cytototoxicity. This review aims to provide up-to-date information about the pharmacology; phytochemistry and clinical safety with toxicity of ***Mallotus philippinensis*** based on scientific literature in order to reveal it's therapeutic and gaps requiring future research opportunities.Major phytochemicals such as diterpeniods, steroids, flavonoids, cardenolides, triterpenoids, coumarin and isocoumarins, especially phenols i.e. bergenin, mallotophilippinens, rottlerin and isorottlerin have been isolated, identified and reported for antioxidant, anti-inflammatory, immunoregulatory activity protein inhibition and cytotoxicity against cancer cell. The present review reveals that Mallotusphilippinensis is a valuable source of medicinally important natural molecules and provides convincing support for its future use in modernmedicine.Indicators used in titration show well marked changes of colour in certain intervals of pH. Most of theseindicators are organic dyes and are of synthetic origin. ***Mallotusphilippinensis*** linn belonging to the family: Euphorbiaceae ; it iscommonly called as white Mullberry; toola; tuk; shetu.The present work highlights the use of the acidified methanolic extract of the fruit of ***Mallotusphilippinensis*** as an acid-base indicator inacid-base titrations. Titration shows sharp colour change at the equivalence point. Therefore, this natural indicator is found to be a very useful, economical, simple and accurate for acid base titration.

Introduction

India has some of the world's most biodiverse regions. Biodiversity must be seen in the light of holding ethical value. Man holds great responsibility towards preserving and conserving other organisms. Some ecologists and philosophers feel that we have a moral obligation to preserve organisms with lesser powers. The genus MallotusLour., (Euphorbiaceae) comprises of about 150 species in the world, of which 20 species has been reported from India 1 And species with 2 varieties were reported from Tamil Nadu state. An ethnomedicinal plant, Mallotusphilippensis (Lam.) Muell. Arg., var. philippensis locally known as Kapilapodi has been used medicinally for long time throughout India. The leaves are used externally for parasitic infections, aphrodisiac, skin infections, wound healing 3, 4, 5. Recent work has reported an antifilarial activity of M. philippensis .

Scientific classification--

Kingdom- Plantae, Subkingdom - Tracheobionta
Superdivision- spermatophyta , Division - Magnoliophyta
Class - Magnoliopsida , Subclass - Rosidae
Order - Euphorbiales, Family - Euphorbiaceae
Genus - Mallotus, Species - Mallotusphilippinensis

Biophysical Limits -

Mostly grown at an altitude of 0-1600 m at it mean annual temperature of 16-28º C with mean annual rainfall was 800-2000 mm. Plants will grown mostly in a wide range of soil types, including infertile soils, limestone, acid and rockyland.

Vernacular names and traditional uses -

Vernacular names-

English- Kamala tree. Hindi- Kamala, Sindur, Rohini, Kambhal. Bengali- Kamala, Kamalagundi. Guajarati- Kapilo. Kannad- Kampillaka, Kunkumadamara. Malayalam- Sinduri, Manjana, Kuramatakku, Kampipala, Ponnagam. Marathi- Shindur, Shendri, Kapila. **Punjabi**- Kumila, Kamal, Kambal, Kamela. Tamil- Kapli, Kungumam, Kurangumanjanatti, Kamala, Manjanai, Kunkumam, Kamala. Telugu- Kunkuma, Chendra-sinduri, Kapila, Vassuntagunda, Sundari, Vasanta, Kumkumamu. Arabic- Kinbil. Assam- Gangai, Puddum, Lochan. Oriya- Bosonto-gundi, Kumala, Sundragundi, Kamalagundi. Pers - Kanbela. Santhal- Rora.

Traditional uses-

According to Ayurveda, leaves are bitter, cooling and appetizer. All parts of plant like glands and hairs from the capsules or fruits are used as heating, purgative, anthelmintic, vulnerary,detergent, maturant, carminative, alexiteric and useful in treatment of bronchitis, abdominal diseases, spleen enlargement and if taken with milk or curd (yoghurt), it can be quite useful for expelling tapeworms . Kamala or Kampillakah is also used as an oral contraceptive. The powder and a few other parts of Kamala are also used in external applications to promote the healing of ulcers and wounds. They are used to treat parasitic affections of the skin like scabies,ringworm and herpes.

Economic Potential and Uses-

Kamela dye: Orange-yellow dye for textiles. The fruit of the tree has a red glandular pubescence which, when detached and comminuted, is known as kamala powder.

- On dyeing textiles such as silk and wool, kamala powder produces an attractive bright orange colour but this gradually fades on exposure to the sun.
- Kamela has been known as a remedy for *tapeworm* among European and American physicians, for only a few years, though long known and employed for this purpose in India
- The Indian kamela was used as an adulterant for Wars during one time. It is also used in adultering the dye prepared from *Bixaorellana* by combining the dye from the seeds with that prepared from the roots and leaves.
- Dye is used as a sindhur or kumkum by women in India.

- This dye is also used for colouring oils, soaps, ice-cream and drinks.
- Rottlerin and its tri- and penta-potassium derivatives are employed for colouring lemonades, lime juice and other beverages.
- Roots also yield a red dye.

Chemistry-

The Kamela powder is said to be aromatic; is but slowly wetted by water and yields but little colour even to boiling water, colouring it pale yellow. In the presence of alkaline carbonates and caustic alkalies, especially the latter, it forms deep red solutions. The extract prepared with soda imparts to silk a fine and durable fiery-orange colour, without further addition or the use of mordants; with cotton, on the other hand, it does not produce a good colour. The natural dye stuff contains 3.49 percent of water 78.19 percent resinous colouring matters, 7.34 albuminous substances, 7.14 cellulose and 3.84 ash, besides small quantities of volatile oil and a volatile colouring matter. The liquid distilled from the alcoholic extract has a yellow colour and the odour of the original substance. The concentrated etherialextrat of the colouring matter deposits a yellow crystalline substance called rottlerin. The extract, prepared with boiling alcohol, deposits, on cooling, non-crystalline flecks of a substance having the composition C_20_3 H_4O_4.

Common Adulterants -

Glandular hair powder of M. philippinensis is commonly adulterated with Annato dye (Bixaorellana Linn.), ferric oxide, brick dust and ferruginous sand. Casearіatomentosa (stem bark powder), Carthamustinctorius (flower powder), Ficusbenghalensis (fruit powder) and Flemingiamacrophylla (hairs of fruits) are also reported to be used as adulterant or substituteofKampillaka. .

Chemical constituents-

Major phytochemicals present in this genus contains different natural compounds, mainly phenols, diterpeniods, steroids, flavonoids, cardenolides, triterpenoids, coumarin and isocoumarins and many more to discover. Present knowledge about this endangered species of medicinal plant is still limited with respect to its phytochemistry and biological activity.However some researchers have contributed towards isolation of some novel constituents and its activity. One of the major chemical constituent i.e. rottlerin of M.philippinensis is listed below with its chemical structure and its major biological activities along with other phytochemicals.Rottlerin, yellow and red resins, wax, and a yellow crystalline substance, tannic acid, gum, and volatile oil.

Material And Methods-

The pomegranate fruit was taken and peeled the epidermis. The arils were collected from fruit and the juice was extracted by separating the seeds manually. The experimental work was carried out by using the same set of glasswares for all type of titrations. As the same aliquots were used for both titrations i.e. titration by using standard indicators and pomegranate extract, the reagent were not calibrated. The equinormal titration was performed using 20 mlof titrate with three drops of indicator and the assay of sodium bicarbonate was carried out.using three different molar strength of acids and alkalies viz. 0.1, 0.5, & 1.0 M.

For all types of titrations equivalence pointobtained by the fruit extract either exactly coincided orvery closed with the equivalence point obtained by the

standard indicators. This represent the usefulness of fruitextract as an indicator in acid base titrations.Analytical grade reagents i.e. hydrochloric acid(HCL), sodium hydroxide (NaOH), acetic acid(CH3COOH), ammonia (NH3), phenolphthalein andmethyl red were procured from the AppasahebBirnaleCollege of Pharmacy, Sangli. Reagents and volumetricsolutions were prepared as per Indian Pharmacopoeia.The fresh fruits were collected from a gardenlocated in Mahabaleshwar (M.S.) and authenticated atDepartment of Botany, Wellingdon College, Sangli(M.S.). The fruits were cleaned and cut into small pieces.100 gm of these pieces were macerated with 150 ml ofsolution containing 9 parts of methanol and 1 part ofdilute hydrochloric acid for 45 min5. The extract waspreserved in tightly closed container and stored awayfrom the direct sun light.

Result and discussion:

Table--3

Extract	Titrate	Titrant	Colour change
1)Alcoholic extract	a) H2SO4	NAOH	Yellowish Red--Colourless PH Range (4-6)
2) Aqueuos Extract	a) NAOH	HCL	Faint red --colourless PH Range (5.5-6.7)
3) Alcohol;ic Extract	b) NAOH	HCL	Yellow--colourless PH Range (4.2-6.2)
4) Alcoholic Extract	a) KOH	CH3COOH	Orange --Pale yellow PH Range (4.4-6.5)
5) Aqueos Extract	b) KOH	CH3COOH	Yellow--faint brown PH Range (2.4-5.5)
6) Alcoholic Extract	a) NAOH	CH3COOH	Orange--Pale Yellow PH Range (3.5-6.3)
7) Alcoholic Extract	a) KOH	HNO3	Faint brown--YelloW PH Range (4.5-6.8)
8) Alcoholic Extract	a) KOH	HCL	Brownish Yellow-Yellow PH Range (5.4-6.7)

The fruit was screened for its use as an acid baseindicator in acid base titrations and the result of this screening were compared with the results obtained by standard indicators methyl red, phenolpthalinThe results of the screening for strong acid
strong base (HCL &NaOH), strong acid weak base (HCL & NH3), weak acid strong base (CH3COOH &NaOH) and weak acid weak base (CH3COOH & NH3) are listed in Table 3. The table represents mean of five titrations + standard deviation. The screening was carried
out using three different molar strength of acids and alkalies viz. 0.1, 0.5, & 1.0 M. For all types of titrations equivalence point obtained by the fruit extract either exactly coincided or very closed with the equivalence point obtained by the standard indicators. This represent the usefulness of fruit extract as an indicator in acid base titrations. Its use in weak acid weak base was found to be more significant over standard indicator as it gives sharp colour change in a narrow pH range. The results obtained showed that the routinely used indicator can be replaced successfully by fruit extracts.

Chemical Tests-

Table 1-

Sr..no.	Name of test	Ethanolic extract
1	**Test for Carbohydrates-**	
	a)Test for redusing sugars-	Brick red color ppt.
	1)Fehlings test-fehlings reagent A and 1ml fehling B solutions,boil for 1mint	Redusingsuger present
A	2) Benedict's test---	
	Mix equal volume of Benedict's reagent and test solution + heat it	Red colour obtained Redusingsuger present.
2	**Test for Proteins**--	Violet color
	a)Biuret test(general test)-- To 3ml T.S.add 4% NaOH+FEW drops of 1% Cuso4+solution.	Protein present.
	b) Millions test(for proteins)--	
	Mix.3ml T.S.with 5ml millions reagent.Whiteppt.Warm.	Red colour Protein present

3	**Test for non -redusing polysaccharides (starch)--**	
	a) Iodine test--Mix 3ml test solution and few drops of dillute iodine solution	Blue color Starch present
	b) Tannic acid test for starch;--- With 20% tannic acid	Ppt formed Starch present.
4	**Test for amino acids-**	
	a)Test for tyrosine--Heat 3ml T.S.& 3 drops millions reagent .solution .	Dark red color Amino acid present.
	b) Test for cysteine--To 5ml T.S.add few drops of 40% NAOH & 1%Lead acetate solution.boil solution	Black ppt Amino acid present.
5	**Test for steroid--**	
	a)Liebermann Burchard reaction-- Mix 2ml extract ,add 2ml chloroform &2ml conc.H2SO4.	Green color Steroid present.
	b) Liebermann's reaction---	
	Mix 3ml extract with chloroform and add 1-2ml aceteicanhydride.heat&cool.+conc.H2SO4.	Blue color Steroid present.
6	**Test for glycosides--**	
	a)Test for deoxysugers (keller-killiani test)---	
	To 2ml extract ,add glacial acetic acid ,one drop 5% fecl3 and conc.H2SO4	Reddish brown junction of 2 liquid layers and upper layer appears bluish green.
		-Glycosides present.
7	**Test of flavonoids--**	
	a) Shinoda test --to dry powder or extract add 5ml 95% ethanol/t-butyl alcohol,few drops of conc.HCL and 0.5 gmagnesium turnings.	Orange,pink,red to purple color appears. Flavonoids present.
	b)Sulphuric Acid Test---	
	On addition of sulphuric acid (66% or 80%)flavones and flavonols dissolve into it & give deep yellow solution.	Chalcones&auronesgives red solution.
8	**Test of Alkaloids--**	
	a) Dragendorff's test-	
	To 2-3 ml filtrate ,add few drops ,dragendorff's reagent	Orange brown ppt. Alkaloid present
	b)Mayer's test-	
	2-3ml filtrate with mayer's reagent gives ppt.	Ppt. Alkaloid present.
	c)Hager's test --	
	2-3 ml filtrate with Hager's reagent	Yellow ppt. Alkaloid present
	d)Wagner's test--	
	2-3 ml filtrate with few drops Wager's reagent.	Reddish brown ppt. Alkaloid present
9	**Test for tannin and phenolic compounds--**	
	a) 5% fecl3solution	Deep blue-blank colour. Tannic acid present.
	b)Lead acetate solution	White ppt. Tannic acid present
	c)Bromine water	Decolouration of bromine water. Tannic acid present

10	**Test for organic acids --**	
	a) Calcium chloride test-	
	To 2 ml test solution and few drops of 5% cacl2 solution	Oxalic acid present

THIN LAYER CHROMATOGRAPHY--

A. **Stationary Phase**-- SilIica gel g

B. Mobile Phase-- -1. Chloroform **:** Benzene : Acetic acid (6 : 7 : 8)

2. Acetic acid : Ethyl acetate : Benzene (5 : 6 : 7)

C. Detector Used in TLC--- Iodine Chamber.

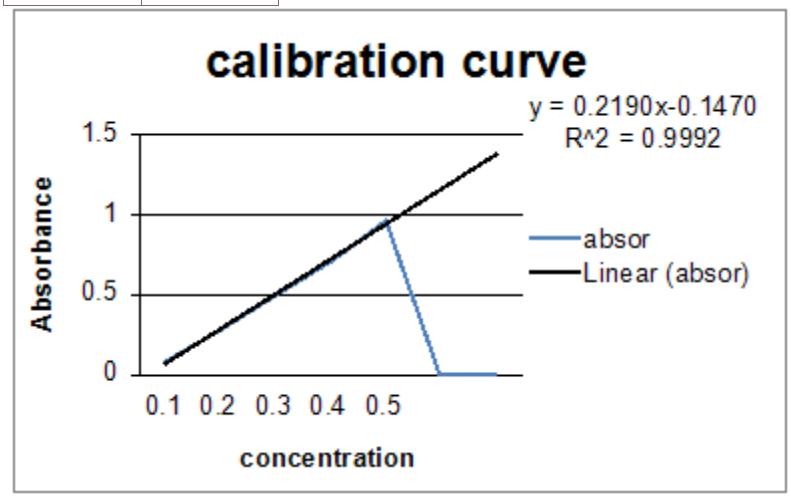
R.F. Value== Distance traveled by solute
Distance travelled by Solvent

1. **R.F. Value** == 4.4/7.6 = o.57
2 **R.F Value** == 4.5/ 6.5 = o.65

Calibration Curve of Mallotusphilippinensis

Table 2- RPM - 270

concen	absor
0.1	0.08
0.2	0.29
0.3	0.5
0.4	0.72
0.5	0.96



Conclusion:

The results obtained in all the types of acid base titrations lead us to conclude that it was due to the presence of flavonoids sharp colour changes, which occurred at end point of titrations. At the end of it states that fruit **Mallotusphilippinensis** extract as an natural indicator in all types of acid base titrations.

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