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PHYTOCHEMICAL STUDIES ON SYZYGIUM CUMINI: A TRADITIONAL DRUGS FOR DIABETES

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ABSTRACT

Herbal medicines as the major remedy and traditional system of medicine have been used in medical practices since antiguity. The phytochemical analysis of the medicinal plants are important and have great interest in both research institutes and pharmaceutical companies for the production of new drugs for curing of various diseases. The present study was aimed to investigate the presence of various phytochemicals from the ethanolic and aquous extracts of leaf, stem bark, flower,root and seeds of *Syzygium cumini*. Five parts viz. leaf, stem bark,flower, root and seeds extracts of *Syzygium cumini* were investigated for their phytochemicals and HPTLC analysis. The plant extract contains carbohydrates, proteins, alkaloids, flavonoids, tannins, steroids, tri-terpenoids, phenol and saponin. The present study provides evidence that the plant contains medicinally bioactive compounds & this justifies the use of the plant species as traditional medicine for treatment of diabetes. Additional work is encouraged to elucidate the possible mechanism of action of these extracts.

Introduction:

Diabetes mellitus is a global epidemic and one of the leading causes of morbidity and mortality. Additional medications that are novel, affordable and efficacious are needed to combat against this rampant disease. Medicinal plants have been used in primary health care over many centuries before the advent of modern medicine. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008)[1]. *Syzygium cumini* (L.) is belonging to the family Myrtaceae (having perfume-Greek Myron =perfume). It is indigenous to India and Indonesia, but it naturalized in Thailand, the phillippins and Madagascar and cultivated throughout Afrika & tropical America.

The plants are reported to contain vitamin C, gallic acid, ellagic acid ,tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components (Martinez and Del, 1981, Wealth of India Raw materials 1976)[2,3,4]. Leaves of *Syzium cumini* containing acylated flavonol, glycosides, essential oils[5] (quercetin, myricetin, myricitin, myricetin 3-O-4-acetyl-L-rhamnopyranoside[6], triterpenoids[7], esterase, galloyl carboxylase and tannin. The stem bark is contain betulinic acid, friedelin, epi-friedelanol, -sitosterol, eugenin and fatty acid ester of epi-friedelanol8, -sitosterol, quercetin kaempferol, myricetin, gallic acid and ellagic acid[9], bergenins, flavonoids and tannins[10]. The roots are rich in flavonoid glycosides and isorhamnetin 3-O-rutinoside[11]. Seed oil contains lauric, myristic, palmitic, steric, oleic, lonoleic, malvelic, sterculic and vernolic acid[12].

The plants were pharmacologoically proves to posses hypoglycaemic, antibacterial, anti-HIV activity and anti-diarrhea effects (Bhuiyan et al., 1996, Indira and Mohan, 1993 and Ravi et al., 2004)[13,14,15]. Leaves and barks of *Syzygium cumini* have anti-inflammatory activity (Muruganandan et al., 2001)[16]. The aqueous and methanolic extract of *Syzgium cumini* (seed) and shows α-amylase inhibition[17]. Leaves have been also used in traditional medicine as a remedy for diabetes mellitus in many countries (Rahman and Zaman 1989)[18]. The bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and used for the treatment of sore throat, bronchitis, asthma, thirst, biliousness, dysentery and ulcers. It is also a good blood purifier. The fruit is acrid, sweet, cooling and astringent to the bowels and removes bad smell form mouth, biliousness, stomachic, astringent, diuretic and antidiabetic19. Aquas suspension of seed kernel of S.cumini showed hypoglycemic effect in normal rabbits and alloxan induced diabetes in rat. Seed powder showed hypoglycemic effect in strepto zotocin induced diabeties and lowered cholesterol free fatty acid and triglyceric level[12].

The plant was used for long time in Indian tradition, but it wasn't studied to determine its chemical composition. The plants have complicated composition and the phytochemical studies can be performed using chromatographic and spectral methods that can separate and characterize the different compounds from complex matrix. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structure determination of Phytochemical. The present study deals with phytochemical investigations and TLC identification analysis of leaf, stem bark, seed flower and root of Syzygium cumini (L.)

Research Elaboration:

Plant material

Fresh the plant specimens (leaves, root, seed , flower and stem bark) of Syzygium cumini (L.) were collected from herbal garden of Arogyadham campus, Deendayal research Institute Chitrakoot, Satna, MP, India and authenticated by taxonomist. A voucher specimen no. 102 is kept at herbarium of research lab in University campus, Chitrakoot for further reference. The All chemicals and reagents used including the solvents were of analytical grade.

Preparation of extracts

All the plant specimens were washed thoroughly with normal tap water followed by sterile distill water and air dried at room temperature for one week to get consistent weight. The dried samples were crushed to powder using grinder. Powder were stored in air tight container bottle 10 gm of each sample were extracted with 100 ml of methanol and water for using soxlet extraction.

The mixtures were evaporating under reduced pressure using rotary vaccum evaporator (Buchi type). And stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemicals.

Preliminary Phytochemical Screening

The phytochemical tests of the ethanol and water extracts of Syzygium cumini (L.) were carried out to identify various

phytocostituents using with standard procedures20,21,22 for determining the presence and/or absence of phytochemicals.

Quantitative Analysis of phytochemicals

Determination of total polyphenol

The concentration of phenolics in methanol fraction of *Syzygium cumini* (L.) was determined with the Folin–Ciocalteu's reagent (FCR) using specrophotometric method[23,24]. Each sample (1 ml) was mixed with 0.5mL FCR (diluted 1:10, v/v) followed by 2 ml of sodium carbonate (20.00%, v/v) solution. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 ug/ml) were prepared. The absorbance was measured at 660 nm for test and standard solutions against the reagent blank at 765nm after incubation at room temperature for 90 min. Results were expressed as mg of GAE/gm of extract.

Determination of total proteins:

Extraction of Protein from sample

Extraction is carried out with buffers used for the enzyme assay. Accurately weighed 500 mg of the sample were ground well with a pistle and mortar in 5-10 ml of the buffer. Centrifuged (SORVALL RC 5B plus) 20 minutes at 10,000 per rpm and collected supernatant used for protein estimation[25].

Standard Protein solution

Weighed accurately 50 mg of bovine serum albumin (fraction V) and dissolved in distilled water and the volume was made up to 50 ml in a standard flask. Diluted 10 ml of the stock solution to 50 ml with distill water in a standard flask. 1 ml of this solution contains 200 µg protein.

Estimation of protein

Pipette out 0.2, 0.4, 0.6, 08 and 1 ml of the working standard into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes. The volume was made to 1ml in all the test tubes. A tube with 1 ml of water serves as the blank. Added 5 ml of Alkaline copper solution to each tube including the blank. Mixed well and allowed standing for 10 minutes. Then added 0.5 ml of freshly prepared Folin-Ciocalteau reagent mixed well and incubated at room temperature. Kept in the dark for 30 min. A blue color is developed. The absorbance was measured at 660 nm by UV spectrophotometer and calculated the amount protein in the sample. By plotting a standard graph and expressed the amount of protein in mg/gm of sample.

Determination of Total Saponin:

Determination of total saponin was done using anisald ehyde reagent. Sample solution was prepared in water. For total saponin estimation 500 µl of sample, 500 µl of 0.5% anisaldehyde reagent were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60 °C. After 10 min tubes were cooled and absorbance was taken at 435 nm by UV spectrophotometer. The amount of saponin was calculated as saponin equivalent from the calibration curve of standard saponin (100-1000 µg/ml) [26].

Determination of alkaloids:

Calibration curve

The calibration curve was obtained with bismuth nitrate pentahydrate stock solution. Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml of stock solution into separate 10ml standard flask and diluting to volume with distilled water. A 1ml amount of this solution was taken and 5ml thiourea solution was added to it the absorbance value of the yellow solution was measured at 435 nm against colorless reagent blanks[27].

Assay for alkaloids

A 5 ml amount of the extract/solution was taken and the pH was maintained at 2-2.5 with dilute HCL. A 2 ml amount of Dragendorff's Reagent was added to it, and the precipitate formed was centrifuged. The centrifuged was checked for complete precipitation by adding DR. after centrifugation the centrifuge was decanted completely and meticulously. The precipitate was further washed with alcohol. The titrate was discarded and the residue was then treated with 2ml disodium sulfide solution. the brownish black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2mlconcentrate nitric acid with warming if necessary this solution was diluted to 10 ml in a standard flask with distilled water.1ml was then pipetted out and 5 ml thiourea solution was added to it, the absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The amount of bismuth present in the solution was calculated using calibration curve. The amount of bismuth corresponds to the amount of alkaloids present.

High performance thin layer chromatography:

The methanolic extracts of the samples T^1 (leaf) T^2 (seed) and T^3 (stem bark) T^4 (standard marker) T^5 (flower) T^6 (root) of *Syzygium cumini* (L.). were subjected to HPTLC fingerprint profile to check the presence of different phyto-constituents. HPTLC Fingerprinting was carried out on pre-coated silica gel HPTLC aluminum plates 60F254 (10 cm×10 cm, 0.2mm thickness, 5-6 µm particle size, E-Merck, Germany) by using CAMAG HPTLC System (Switzerland). 4 µL and 6 µL of each of the extract were spotted as bands of 8 mm width by using a LINOMAT 5 Sample Applicator fitted with a 100 µL Hamilton Syringe. The plates were developed using *Tolune: ethyl acetate* (7:3) as a mobile phase in CAMAG twin-trough chamber lined with filter paper and pre-saturated with 10 ml mobile phase. The resulted plates were dried in air and photo documentation was done at ultraviolet light at 254nm, 366nm and day light using CAMAG REPROSTAR 3 equipped with WINCATS Software. Numbers of bands, color of separated compound and Rf values were recorded[28,29].

Result and Discussion:

In present study the leaves, stem bark, seed and root of *Syzygium cumini* (L.) Linn were evaluated for its phyto chemical aspects.

The results of preliminary phytochemical screening in the methanolic and water extracts of the drugs showed the presence of carbohydrates, flavonoids, alkaloids, resin, saponins and tannins, results were summarized in Table-1. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity.

The quantitative analysis of phenolic contents, protein, saponin and alkaloid were carried out using spectrophot ometer and results are given in Table-2. The total phenolic contents in the examined plant extracts is expressed in terms of gallic acid equivalent (the standard curve equation: Y=1.77186x+0.07036, r2=0.99989). The concentration of total phenols was measured 2.586, 6.4525, 1.9837, 1.2341, 6.495 ug/ml for leaf, stem bark, seed, flower and root respectively. The protein contents in the examined plant extracts is expressed in terms of BSA equivalent (the standard curve equation: Y=0.05346x + 0.1614, r2=0.99843).The concentration of protein was measured 2.196, 3.5166, 2.389, 2.3562, 1.697 ug/ml for leaf, stem bark, flower and root, seed, flower respectively. The Saponins contents is expressed in terms of Saponins equivalent (the

standard curve equation: Y=0.07150x + 0.01277, r2=0.97804). The concentration of Saponins was found 0.339, 0.283, 0.282, 0.435 and 0.250 ug/ml for leaf, stem bark, seed flower and root respectively. Similarly the alkaloid contents is expressed in terms of mg of Bismuth nitrate/g of extract equivalent (the standard curve equation: Y=0.99892x + 0.19872, r2=0.99693).The concentration of alkaloids was found 0.174, 0.236, 0.319, 0.318, 0.457 ug/ml for leaf, stem bark and seed, flower, root respectively.

The results from HPTLC Fingerprint observed under ultraviolet region at 366nm and after derivatization with 5% methanolic H2SO4. The color of the band resolved and their Rf values were tabulated in Table-3.

A simple qualitative HPTLC analysis was performed and Rf values were recorded. HPTLC Plate shows one bands at 366 Rf 0.91 for test sample T1 contains one bands at Rf, 0.91, T2 observed eight spot at Rf 0.6,0.10 (red), 0.24 (red), 0.54 (blue), 0.62 (red), 0.66 (red) 0.83,0.91 (red). In test sample T3 observed seven spot 0.6 (red), 0.91 (red), 0.91 and T4 observed five spot Rf value 0.8 red, 0.34, golden yellow, 0.38 blue 0.61 blue ,0.79 (sky blue). T5 observed six spots 0.6 red, 0.79 blue, 0.91 red, 0.91 (red) and T6 observed five spots 0.91 (red), 0.57, 0.6 (red), 0.79 (red) and 0.91 (red). After derivatization with 5% methanolic H2SO4 the spots of T1 0.91, T2 0.10 (pink), 0.57 (greenish yellow), 0.84 (pink), 0.91 (red), T3 0.57 (green yellowish), 0.91, T4 0.8 (red), 0.33 (green), 0.57 (green), 0.78 (green), 0.91 (red), T5 0.57(green), 0.91 (red), 0.33 (green), 0.57 (green), 0.78 (green), 0.91 (red), day light have one spot 0.57 and T6 0.91 (red), 0.57, 0.57 (green), 0.91(red), 0.57 were resolved under 366 nm. The results of Rf values and colors of the resolved bands were shown in Table-5 & Plate-1(Fig- a, b & c). The current findings can be used as a reference standard for identification ellagic acid. Marker based identification and pharmacological studies enhance its therapeutic potential to maintain diabetes mellitus. The positive health effects may be due to high contents of certain phenolic compounds in plant-derived foods. Recently, phytochemicals and their effects on human health have been intensively studied. In particular, a search for antioxidants, hypoglycemic agents, and anticancer agents in vegetables, fruits, teas, spices and medicinal herbs has attracted great attention.

Conclusion:

Syzygium cumini (L.) is extensively used in the traditional system of medicine for treatment of number of diseases and considered as an important medicinal plant. As per literature survey very less work has been reported on this variety. From the present investigation various standardization parame ters such as Phytochemical analysis were carried out as per WHO/Ayurvedic Pharmacopoeial standards. In the present study, most of the biologically active phytochemicals such as flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides and tannins were found to be present in the ethanolic extracts of different parts of the *Syzygium cumini* plant. The medicinal properties of Syzygium cumini plant extracts may be due to the presence of above mentioned phytochemicals.

Presence of various phytoconstituents can serve as basis for screening of different pharmacological activities, investigation and further research. Total phenol, protein, alkaloid and saponin contents were investigated in the drug. The pharmacological action of these plant drugs will be determined by the nature of these chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. The developed HPTLC chromatograph may be used for quality control purpose.

Current findings ascertain the claimed medicinal or therapeutic effects of plants secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs.

Table-1. Phytochemical Analysis

Phyto-chemicals	Tests	Leaf		Bark		Seed		Flower		Root	
Carbohydrates		WE	AE	WE	AE	WE	AE	WE	AE	WE	AE
	Anthrone test								+	–	
	Fehling test	+	-	-	-	+	-	-	+	_	-
Resin	Acetone	+				+			-	-	
Saponins	Foam tests	+				+			+	+	
Alkaloids	Dragendorff's test,	+	+	-	-	+	-	-	+	+	-
	Wagner's	+	+		-	+	+		+	+	+
Steroids	Salkowski tests	+	-	-	-	+	-	-	+	+	-
Flavanoids	Magnesium test	+	+	+	+	+	+	+			+
Tannins	Shinoda test 5% FeCl3	+	+	+	+	+	+	+	_	-	+
Amino acids and Proteins	Biurate test	+	+	+		+		+	+	+	+

WE= water extract, AE= alcohol soluble

Table-2. Quantitative estimation of Phytochemical Analysis

SN.	Phytochemical		Concentration in ug/ml				Unit
		leaf	Stem Bark	seed	flower	Root	
1	Polyphenol	2.586	6.4525	1.9837	1.2341	6.495	mg of GAE/g of extract
2	Protein	2.196	3.5166	2.389	2.3562	1.697	mg of BSA/gm of extract
3	Saponin	0.339	0.283	0.435	0.282	0.250	mg of Saponins/g of extract
4	Alkaloid	0.174	0.236	0.319	0.2321	0.457	mg of Bismuth nitrate/g of extract

Table 3. R_f values color of the bands resolved in test solutions of *Syzygium cumini* Linn.

	Mobile phase- Tolune: Ethyl Acetate (9:1)		
	366nm	366nm AD	Day light AD
T1	0.91 (red)	0.91	
T2	0. 6, 0.10 (red), 0.24 (red), 0.49 (blue), 0.62 (red), 0.66 (red),0.83,0.91(red)	0.10(pink),0.57 (green yellow),0.84 (pink),0.91 (red)	0.57,0.61, 0.77,0.91
T3	0.6 (red), 0.91 (red)	0.57(greenish yellow),0.91	0.57
T4	0.8 (red),0.34 (golden yellow), 0.38(blue), 0.61(blue),0.79 (sky blue),0.91 (red)	0.8 (red), 0.33 (green), 0.57 (green),0.78 (green),0.91 (red)	0.57

T5	0.6 (red),0.79 (blue), 0.91(red)	0.57 (green),0.78 (green),0.91 (red)	0.57
T6	0.6 (red),0.79 (blue), 0.91 (red)	0.57 (green),0.91 (red)	0.57

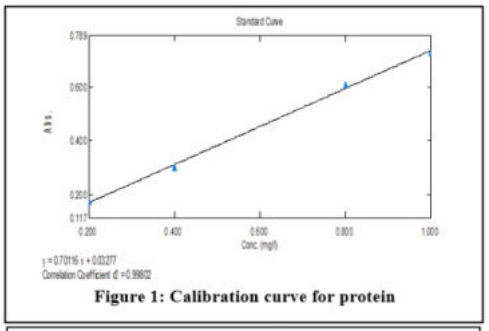


Figure 1: Calibration curve for protein

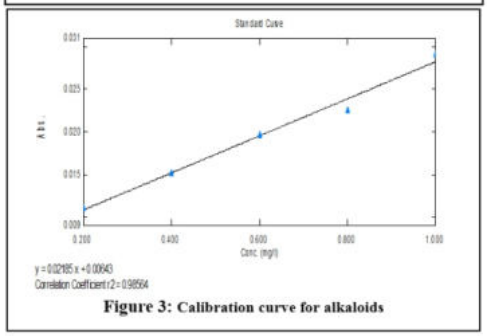


Figure 3: Calibration curve for alkaloids

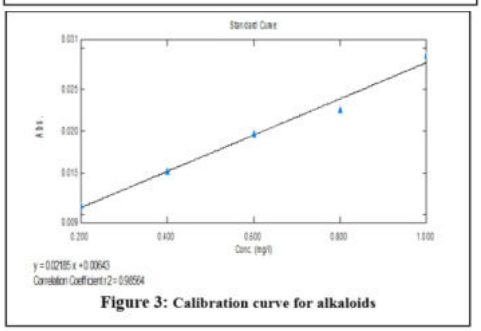


Figure 3: Calibration curve for alkaloids

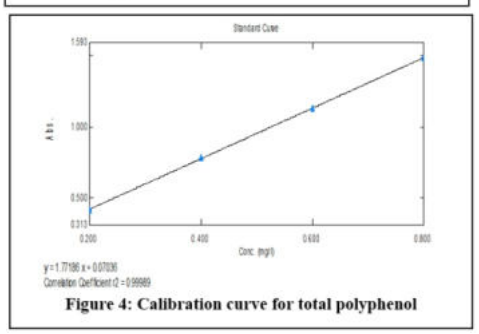
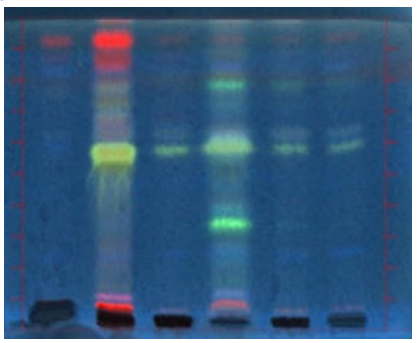


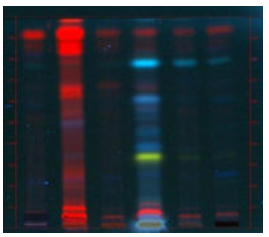
Figure 4: Calibration curve for total polyphenol

Plate: HPTLC Chromatographs of test samples and standard

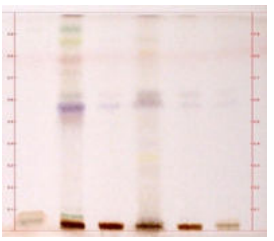


T1 T2 T3 T4 T5 T6

Fig.1. Observed at 254 nm



T1 T2 T3 T4 T5 T6



T1 T2 T3 T4 T5 T6

I=254nm, II=366nm and III=366nm (visible light)

Tracks=T1=leaves, T2 = seed, T3=stem bark,, T4= standard (Ellagic acid), T5= flower,T6=root

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