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RESEARCH ARTICLE

Phytochemical and Pharmacological Investigation of Roots of *Syzygium Cuminii (L)* Skeel

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ABSTRACT
The roots of *syzygium cuminii* (L) skeel was collected in Gulbarga district Karnataka. Dried and subjected for extraction with petroleum ether ,chloroform the residue obtained after the evaporation of chloroform was taken for further study to isolate six chemical constituent present in it (ScRex-3, ScRex-4, ScRex-5, ScRex-6a, ScRex-6b, ScRex-2,)this constituent were subjected for structural elucidation by physical measurement and phyto-chemical study. The pharmacological study describe is restricted only to the antidiabetic activity of chloroform extract the result obtained is compared with standard used for antidiabetic study for the measurement of glucose level in blood. The further study is in progress to isolate the above component in higher quantity and screen them for antidiabetic properties.

KEY WORDS *Syzygium cuminii,* Myrtaceae, Chloroform extract, antidiabetic

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INTRODUCTION:
Eugenia jambolana (Syn.*Syzygium cuminii (L) Skeel*), belonging to the Family: Myretacea Commonly known as ''Jamun'' . Roots were collected during the month of April 2007 from village Nalwar of Gulbarga District (Karnataka) and was identified and authenticated by Department of Botany, Gulbarga University Gulbarga, a voucher (#72) of specimen was submitted to NGSMIPS, Derlakatte.

Uses in traditional medicine:
The therapeutic value of *Syzygium cuminii (L) skeel* Commonly known as ''Jamun'' in the local language has been recognized in different systems of traditional medication for the treatment of different diseases and ailments of human beings[1], Such as leaves and seed are used in treatment of Bronchitis, asthma, thirst, biliousness, dysentery, ulcers The fruit pulp is used in diabetics and also as a blood purifier. Several studies using modern techniques have authenticated its use in diabetes and its complications (nephropathy, cataract, insulin resistance) [2, 3].

Previously isolated classes of constituent:
Fifteen polyphenols and two Acylated flavonal glycoside are isolated from the leaves[5] of *Syzygium cuminii (L) Skeel.* Essential oil and triterpenoids are isolated from the stem and fruits[6]. From flower olinolic acid, triterpenoid, ellagic acid and flavanols are isolated in small amount[7].The literature survive reveals that there is no substantial work has been carried out on roots of *Syzygium cuminii (L) skeel.* Hence effort is made to investigate for the first time the roots of *Syzygium cuminil (L) skeel.*

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MATERIALS AND METHODS:
All the melting points were recorded by Toshniwal melting point apparatus and were uncorrected. IR spectra of the compounds were recorded using the KBr pellet method on a Nicolet Avator 330 FTIR, Perkin-Elmer model 700, IR spectrophotometer. ^{1}HNMR spectra of the compounds were taken on AMX 400 (270 MHZ), EM-360 (270 MHZ) NMR spectrometer using $CDCl_3$ as solvent. Mass spectra were recorded on FAB-MASS. TLC was carried out using Silica-gel G (Merck). Column chromatography was carried out on Silica-gel (Merck, 70-230 mesh) and cellulose powder (Merck, Bombay) as a stationary phase. All the chemicals and reagents used were obtained in high purity either from S.D. fine chemicals Pvt. Ltd., Bombay, India or E – Merck Pvt. Ltd., Bombay, India.

General experimental procedure[4]
The shade dried roots were powdered (3 kg), extracted in succession by maceration, the solvents were used according to their increasing polarity i.e Pet. ether followed by chloroform. The residues obtained from different solvents were kept in desiccators and the phytochemical investigation was carried out with chloroform extract (the residue obtained from chloroform extract is 35 gm) It gave

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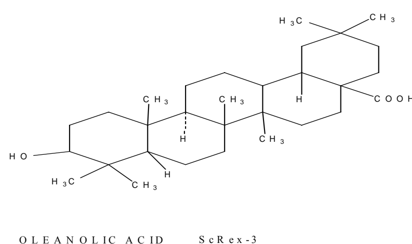
positive test for flavonoides and triterpenoides. The residue (25 g) was triturated in mortar with $CHCl_3$ (10 ml) and adsorbed onto silica gel (20 g). After evaporation of the solvent it was loaded onto a silica gel column (150 g) prepared in petroleum ether (60-80˚C). The column was eluted first with petroleum ether (60-80˚C), petroleum ether (60-80˚C): benzene graded mixtures (95:5, 90:10, 80:20, 70:30, 60:40 and 50:50), then with benzene followed by graded mixtures of benzene: chloroform (95:5, 90:10, 80:20 70:30, 60:40 and 50:50), chloroform and finally chloroform: methanol (95:5, 90:10, 80:20, 70:30, 60:40 and (50:50). The elutions were monitored by TLC (Silica gel-G; visualization by Vanillin-Sulphuric acid reagent heated at 110ºC). 5 ml of identical elutes were collected each time. TLC monitored elutes were combined and concentrated to 5 ml and kept in a refrigerator.

Elution carried out with petroleum ether (60-80˚C): benzene graded mixture (80:20) resulted in getting "one" component (ScRex-1) it was subjected to preparative TLC in the solvent systems *viz*., petroleum ether: benzene (80:20v/v). Two components ScRex-2 and ScRex-3 were isolated from the Benzene graded mixture, the benzene: chloroform (95:5) graded the solvent system for TLC was used *viz.,* Benzene: chloroform (80:20). From chloroform: methanol elutent in different ratios, resulted in isolation of four components first ScRex-4, (80:20), second component ScRex-5, (60:40), third component ScRex-6a and last (50:50) forth component which is designated as ScRex-6b as pure compounds.

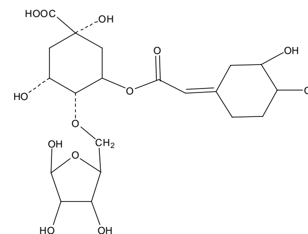
The remaining solvent residue was not analyzed. However the work is going on in this laboratory to analyze the residue.



OLEANOLIC ACID ScRex-3

(3β)-3-hydroxyolane-12-en-28-oic acid

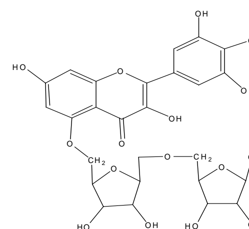
Melting point : 310ºC; Rf value: 0.6 (solvent system; C_6H_6: EtOAc 80:20); IR bands (KBr):3396, 2924, 2854 and 1692 cm^{-1}; ^{1}HNMR ($CDCL_3$) δ 0.99- δ 1.45(number of H,CH CH_2 CH_3 protons); MS m/z (rel.int.): 456 $[M]^+$ ($C_{30}H_{48}O_3$) , 453 (72%), 407 (50%),



CHLOROGENINE GLYCOSIDE ScRex-4

3,4,5-tetrahydroxycyclohexane-carboxalic acid 3-(3,4-dihydroxycinnamate,-O- glycoside

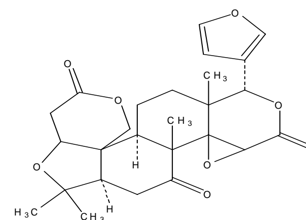
Melting point: 200ºC; Rf value: 0.78 (solvent system; Pet ether:C_6H_6 80:20); IR bands (KBr): 3456,2921, 2850,1693, 1734cm^{-1};^{1}HNMR ($CDCL_3$): δ 0.7- δ 5.5 (number of CH, CH_2 protons) MSm/z (rel.int.):503 $[M]^+$($C_{21}H_{27}O_{14}$), 467(25%), 465(12%), 409 40%) , 39



MYRICETINE GLYCOSIDE ScRex-5

3,5,7-trihydroxy-2-(3,4,5-trihydroxy phenyl)-4H-1-benzopyrone-4-one.5-glycosid

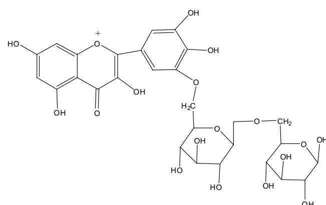
Melting point:300ºC; Rf value 0.5 (solvent system; C_6H_6: EtOAc 80:20); IR bands (KBr):3412 and 1690cm^{-1}; ^{1}HNMR ($CDCL_3$) : δ 0.8- δ 0.48 (number of CH, CH_2 protons); MS m/z (rel.int.):661 $[M]^+$($C_{25}H_{28}O_{10}$), 599(30%), 397 (90%)



LIMONIN ScRex-6a

Limonoic acid di-δ-Lactone

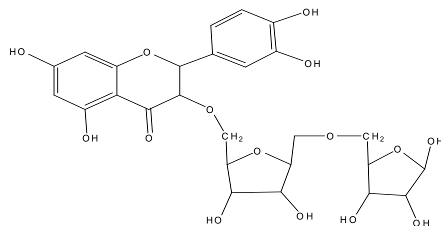
Melting point:298ºC; Rf value 0.36 (solvent system; C_6H_6: EtOAc 80:20); IR bands (KBr):3412 and1694cm^{-1};^{1}HNMR ($CDCL_3$) : δ 0.5- δ 3.8 (number of CH, CH_2 protons); MS m/z (rel.int.): 470 $[M]^+$ ($C_{26}H_{30}O_8$), 471 (60%), 450 (30%), 453 (100%), 407 (10%).



MYRECETIN GENTOBIOSE ScRex-6b

3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-1-benzopyrone-4-one.gentobios

Melting point: 350ºC; Rf value 0.42 (solvent1692cm^{-1}system; C_6H_6: EtOAc 80:20);IR bands (KBr): 3430, 2928,and1692cm^{-1}.HNMR ($CDCL_3$) : δ 0.5- δ 3.9 (number of CH, CH_2 protons); MS m/z (rel.int.) 663: $[M]^+$ ($C_{27}H_{35}O_{19}$),663, 453(90%), 407 (60%), 284 (10%), 248 (70%).



QUERCETIN GLYCOSIDE ScRex-2

3,5,7-trihydroxy-2-(3,4,-diihydroxyphenyl)-4H-1-benzopyrone-4-one,glycoside

Melting point: 360ºC; Rf value 0.8 (solvent system; C_6H_6: methanol. 80:20); IR bands (KBr): 3420, 2928,1692cm^{-1};^{1}HNMR ($CDCL_3$): δ 2.5- δ 5.4 (number of CH, CH_2 protons); MS m/z (rel.int.) 601: $[M]^+$ ($C_{25}H_{28}O_{17}$) 585(50%),601(30%),599 (48%), 575 (70%), 453(35%), 397 (80%)

PHARMACOLOGICAL INVESTIGATION

Acute toxicity studies[9]

The acute toxicity study[8]of chloroform extract was carried out in adult female albino rats by "up and down" method (OECD guidelines 425). The animals were fasted overnight and next day chloroform extract of the root *Syzygium cuminii (L) skeel* dissolved in 0.6 % sodium CMC was administered orally at different dose levels. Then the animals were observed continuously for three hours for general behavior, neurological and autonomic profiles. The observations are tabulated according to 'Irwin's Table'**.** The extract showed no toxic effects. This may be attributed to the fact that the root *Syzygium cuminii (L) skeel* has the food value.

Selection of doses

For the assessment of Antidiabetic activity, three dose levels were chosen in such a way that middle dose was approximately one tenth of the maximum dose during acute toxicity studies and a low dose which was 50% of the one tenth dose and a high dose which was twice that of one tenth dose. (100 mg/kg, 200 mg/kg and 400 mg/kg).

Formulation

According to the above mentioned method chloroform extract at a dose of 200, 400 mg/kg body weight were given to rats as a suspension and was administered orally.

Drugs used

Glibinecalamide: was given to rats at a dose of 5mg/kg body weight, p.o as a refrence standard

Selection of animals

Wister albino rats of either sex weighing between 150 - 200 g were used for the study. The animals were obtained from KSHEMA, Deralakatte Mangalore. These animals were used for both acute toxicity studies and antidiabetic activity.

Antidiabetic activity[9].

The animals were stabilized for 1 week; they were maintained in standard condition at room temp; 60 ± 5% relative humidity maintained was 60 ± 5% and 12 hr light dark cycle. They were given standard pellet diet supplied by Hindustan Lever LTD Co. Bombay and water *ad libitum* throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal out put resulting in the death of the animals. Animals were deprived of food for at least 18 hrs but were allowed free access to drinking water.

Sample collection[10]**.**

Blood was collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tube. Blood was collected in fresh vials containing sodium floride and sodium oxalate as anti coagulant. The plasma was separated in an electric centrifuge at 2000 rev/min for 5 min

Experimental Designs:

Alloxan monohydrate (150 mg/kg, body weight) was dissolved in normal saline and injected intraperitoneally to Wister albino rats weighing 150-200 g. After 18 hrs of fasting to induce hypoglycemia[11]. Alloxan was administerd .The blood glucose levels were monitored after 72 hrs of alloxianization. The rats having blood glucose level above 300 mg/dl of blood were selected for the study.

Five groups of six animals each received the following treatment.

Group 1: Control (2ml distill water orally)
Group 2: Diabetic control (Alloxan 150mg/kg i.p)
Group 3: Diabetic + Extract: (200mg/kg, orally)
Group 4: Diabetic + Glibenclamide (5mg/kg, orally)
Group 5: Diabetic + Extract (400mg/kg orally)

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After 72 hrs the animals in all the group were subjected to serum glucose estimation by withdrawing 0.5 ml of blood from the retro orbital vein puncture was taken into the centrifuging cuvette and centrifuged at 2000rpm and removed after 5 minute. From that 2µl of serum was collected by micropipette and 2ml of standard glucose (Agappe Diagnostics Ltd) was added in a 3 ml test tube and kept aside for 15mins. The blood glucose concentration was estimated in spectrophotometer at 505nm

RESULTS AND DISCUSSION:

Table-1. The following table shows the Antidiabetic effect of chloroform extract of roots of S*syzygium cuminii (L) Skeel*:

Group	2nd Day	3rd Day	7th day
Control	1.171± 0.444	2.283±0.373	3.460±0.649
Alloxan+ Glib 5mg/kg	18.97± 0.87** (8.65)	38.92± 1.543** (10.25)	60.964± 0.548*** (12.65)
Alloxan + extract (200 mg/kg)	11.611± 0.331 (6.25)	21.90± 0.580 (12.75)	43.981± 0.720 (14.80)
Alloxan + extract (400 mg/kg)	14.844± 1.209** (6.75)	23.90± 0.44*** (8.25)	49.84± 0.864** (10.65)

P< 0.01=** compared with control; P< 0.001=*** compared with control

Oral administration of two dose levels i.e., 200mg/kg, 400mg/kg, have shown dose dependence i.e., (11.611%, 21.99%, 43.98%) and (14.844%, 23.905%, 49.842%) respectively. The Antidiabetic activity was highly significant on seventh day of treatment with the effective dose i.e., 400mg/kg body weight.

Table-2. Showing the reduction in body weight of rats during the treatment with chloroform extract of roots of Syzygium *cuminii (L) Skeel*:

Group	2nd Day	3rd Day	7th day
Control	12.42± 1.27 (6.75)	22.47± 1.01 (8.25)	34.23± 0.90 (10.65)
All+ Glib	6.272± 0.997 (8.65)	14.60± 1.402 (10.25)	5.854± 1.512 (12.65)
All + extract (300 mg/kg)	12.87± 1.836 (6.29)	17.843± 1.406 (9.29)	10.731± 1.988 (3.87)
All + extract (400 mg/kg)	16.24± 0.56** (7.01)	19.673±0.798** (12.70)	11.46± 0.952** (5.40)

P< 0.01=** compared with control; P< 0.001=*** compared with control

Significantly reduction in body weight is observed with chloroform extract at two dose level (12.87%, 17.84%) (16.24%, 19.63%) on second and third day respectively.

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REFERENCE:

1. Sagarwat H, Mann AS, Khare MD, pharmacological potential of *Eugenia Jambolana*.A review.PHCOG MAG;2006:96-105
2. Kirtikar KR, Basu BD, Indian medicinal plant .vol.II, Delhi. Periodical express;1975:1052-53.
3. Bhattacharejee SK, Hand book of aromatic plant. Pointer publisher; 1999:195-197.
4. Nadakarni KM, Indian mataria medica .Vol.I, popular book deportment Bombay; 1954:516-18.
5. Mahmoud II, Marzuk MS, Moharram FA, El-Gindi MR, Hassan AM, Acylated flavonal glycoside from *Eugenia Jambolana* leaves, Phytochemistry; 2001:58(8),1239-44.
6. Craveiro AA,Androde CHS,Matos FJA,Alenkar MIL,.essential oil of Eugenia Jambolana, Journal of natural product;1983:46(4),591-92.
7. Nir AGR, Subhramanyan SS, Chemical examination of flower of Eugenia jambolana ,J.Sci.industry.Res;1974:21B,457-458.
8. Timbola AK, Szpoganicz B ,Banco A, Monache FD, Pizzolatti MG, New flavanol from the leaves of *Eugenia Jambolana*, Fitotearpea;2002:73,174-76.
9. New OECD 425 guidelines for testing of animal; 2001dec:1/26,1-6.
10. Turner RS, Screening method in pharmacology. New York. Academic Press; 1965,:26-35,302-4
11. Trane M. antihyperglycemic activity of bark of Syzygium cuminii .J.Ethno.Pharmacology;2005:132-135.
12. Trivedi NA,Mazumdar B, Bhatt JD, Hemavati KA, effect of Shilajit on blood glucose and lipid profile in alloxan induced diabetic rat. Indian journal of pharmacology;2004:36(6) 373-376.

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