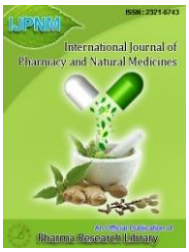

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A study on in vitro Anti Diabetic activity of betasitosterol and it's active isolation identification and characterization from *Mutingia calabura*

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A B S T R A C T
The crude extract of *Muntingia calabura* (*M. calabura*) was subjected to preliminary phytochemical screening, revealing the presence of steroids, flavonoids, saponins, tannins, and carbohydrates, among other compounds. The aqueous extract yielded the highest concentration of these bioactive constituents. High-Performance Liquid Chromatography (HPLC) analysis identified β-sitosterol (46.33%) and stigmasterol (53.67%) as major phytosterols in the extract. The study further investigated the effect of β-sitosterol on GLUT-4 expression and glucose uptake in C2C12 myotube cells. Results demonstrated significant upregulation of GLUT-4, enhancing glucose uptake and suggesting a potential role in glucose homeostasis. Additionally, β-sitosterol also influenced GLUT-6 expression, indicating a non-specific enhancement by the plant-derived compound. These findings suggest that *M. calabura* possesses anti-diabetic properties through its phytosterols, which stimulate GLUT-4 translocation to the cell membrane and may independently or synergistically enhance its epigenetic expression. The observed effects on GLUT-4 translocation imply the potential of *M. calabura* in correcting defects in insulin-mediated glucose transport, making it a promising candidate for diabetes therapy. Furthermore, the modulation of GLUT protein expression at the genetic level by β-sitosterol and structurally similar compounds such as stigmasterol suggests a broader regulatory role in glucose metabolism. This study highlights the therapeutic potential of *M. calabura* in managing both insulin-dependent and insulin-independent diabetes mellitus.

Keywords: *Muntingia calabura*, β-sitosterol, High-Performance Liquid Chromatography, anti-diabetic

A R T I C L E I N F O

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C o n t e n t s

1. Introduction

Diabetes mellitus, a metabolic disorder affecting millions worldwide, is projected to rise to 324 million cases by 2025. Both Type-I (insulin-dependent) and Type-II (non-insulin-dependent) diabetes present significant health challenges, characterized by hyperglycemia, complications such as neuropathy, retinopathy, and nephropathy. While Type I results from insulin deficiency, Type II stems from insulin resistance and inadequate secretion. Additionally, less common forms like heritable and secondary diabetes further complicate management. Modern allopathic

treatments, though effective, often have side effects and high costs, making alternative medicine attractive. Ayurvedic, Siddha, Unani, and Homeopathic systems offer time-tested, natural, and affordable remedies with minimal side effects. Herbal medicines, particularly those from India's rich biodiversity, provide a promising alternative to synthetic drugs. Despite possessing over 45,000 medicinal plant species, India's contribution to the global herbal medicine market remains limited.

2. Materials & Methods

Collection and Identification

Fresh leaves of *Muntigia calabura* were collected were collected at the Vikrama Simhapuri University campus and were authentically identified by Dr. K. Madhavachetty, Department of Botany, S V University, Tirupati.

Preparation of Leaf Extract

The leaves of *Muntigia calabura* were Shade dried and powdered well using a mixer and stored in an airtight container. The leaves, powdered (100g) were taken and subjected to successive solvent extraction (500ml) with Hexane, Chloroform, Ethyl acetate Methanol, and aqueous extracts. The plant extracts were concentrated and stored in an airtight vial for further studies.

Test for Alkaloids:

1.2 g of iodine and 2.0 g of iodide were dissolved in 5mL of sulphuric acid, and therefore the solution was diluted to 100 mL. 10 mL of the extracts of *M. calabura* leaves were acidified by adding 1.5% v/v of HCl and a couple of drops of Wagner's reagent. The formation of yellow-brown precipitate confirmed the presence of alkaloids.

Test for Phenols:

Neutral ferric chloride reagent was prepared and added to the extracts. The looks like of the blue colour showed the presence of blue colour.

Test for Tannins:

The test solutions of the extracts were treated with a couple of drops of lead acetate solution. The formation of white precipitate indicated the presence of tannis.

Test for Flavonoids:

Magnesium ribbon was added to the extracts, and Hcl was added along the edges of the tube. A deep blue color showed the presence of flavonoids.

Test for Saponins:

5 mL of the extracts were taken in several tubes and added a pinch of bicarbonate of soda. The mixture was shaken vigorously and kept for 3 min. A honeycomb-like froth indicated the presence of saponin.

Test for Terpenoids:

2 mL of extract was added to 2 mL of acetic anhydride and concentrated H2SO4. The formation of blue-greenish rings indicated the presence of terpenoids.

Column Chromatography

A column of 25 mm diameter and 900 mm length was taken. Seventy five gram of silica G (100-200 mesh, Sissco, Mumbai) was packed with hexane. The *M. calabura* methanol extract (10-g) was chromatographed over silica column, and therefore the column was eluted with hexane: chloroform (90:10; v/v). The respective fractions were collected and concentrated by flash evaporation and analyzed by TLC.

Characterization of bioactive compounds

The active F4 fractions (1-15) of Rf 0.58 obtained from hexane chloroform partition fraction; obtained through chromatography were characterized by UV-Visible, FT-IR, GC-MS and NMR spectral analysis.

Identification of Bioactive Compound by Analytical Methods

UV- visible spectrometry

The column elutes were observed under Shimadzu 160A UV Double Beam UV- VIS spectrophotometer between 190 to 1100 nm with the solvent blank. 76 About 2 mg sample dissolved in 20 ml methanol was used for recording the spectrum. Fourier transform infrared (FTIR) spectrometry One mg of the substance was mixed by grinding with KBr (Sigma chemicals) and its IR spectrum was scanned within the region of scanned within the range of 4000-400 cm-1 using Perkin Elmer - Spectrum One FT-IR spectrophotometer (Japan).

Mass spectrometry GC-MS analysis:

The sample was prepared within the concentration range 0.2 - 0.5 mg/ml and injected by flow analysis at a flow rate of 10 µl min-1. The recorded mass was within the range 100-500m/z using the spectrum was obtained using Agilent technologies (6890 N) instrument (JEOL GCMATE II) fitted with an electron spray ionization source. Software version 4.0 was used for data acquisition. The positive ion mode, employing a sprig voltage at 3.5 kV, at a source temperature of 80oC, was employed for recording the spectra. The mass spectrum was recorded under electron impact ionization at 70 eV energy.

1H and 13C nuclear resonance spectroscopy:

Thirty mg of the F4 fraction obtained from hexane-chloroform fraction was dissolved in CDCl3 and subjected to 1H and 13C NMR spectral 77 analysis employing a BRUKER 300 MHz instrument. Nuclear resonance (NMR) spectra were recorded on a Bruker 300 NMR instrument operating at 300 MHz for 1H and 125 MHz for 13C at temperature an area from 0 to 12 ppm for 1H and 0-200 ppm for 13C was employed. Signals were mentioned as the internal standard tetramethylsilane.

Screening of *In-Vitro* Anti-Diabetic Activity

Inhibition of alpha-amylase activity (Hansawasdi *et al.*, 2000): The α-amylase inhibitory activity of every extract and phytochemicals determined supported the colorimetric assay using acarbose as a reference compound (19-20). The Starch solution (0.5% w/v) was obtained by boiling and stirring 0.25g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/mL) was prepared by mixing 0.001g of α- amylase in 100 ml of 20 M sodium phosphate buffer (pH 6.9) containing 6.7 mM common salt . The *M.calabura* leaf extracts and isolated phyto- chemicals were dissolved during a respective solvent to offer concentrations for Hexane, Chloroform, ester Methanol, and aqueous extracts are 25 mL, 50 mL, and 100 mL, for ethanol extracts concentrations are 100 µg/mL, 200 µg/mL and 500 µg/mL. 10µg/mL, 50µg/mL and 100µg/mL for isolated phytochemicals. The colour reagent was a solution containing 96 mM 3, 5- dinitrosalicylic acid (20

mL), and 5.31 M sodium potassium tartrate in 2 M caustic soda (8 mL) and deionized water (12 mL). 1 ml of every plant extract and 1 ml enzyme solution were mixed during a tube and incubated at 25°C for 30 min. To 1 ml of this mixture was added 1 ml of starch solution and therefore the tube incubated at 25°C for 3 min. Then, 1 mL of the colour reagent was added, and therefore the closed tube placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 mL water, and therefore the absorbance value determined at 540nm. Individual blanks were prepared for correcting the background absorbance, during this case, the colour reagent solution was added before the addition of a starch a solution then the tube placed into the water bath. The opposite procedures were administered as above. Controls were conducted in a uniform fashion replacing plant extracts and isolated phytochemicals with 1 ml respective solvents. Acarbose solution (at the concentrations of 10µg/mL, 50µg/mL and 100µg/mL) was used as positive control. The inhibition percentage of α-amylase was assessed by the subsequent formula:

$$\text{Inhibition \%} = \frac{(\text{Absorbance of control} - \text{Absorbance of compound}) \times 100}{\text{Absorbance of control}}$$

Inhibition of alpha-glucosidase activity (Kim *et al.*, 2011)
The *M.calabura* extracts to be tested for their inhibitory property of α- glucosidase activity were prepared with various solvents, as mentioned in amylase activity. The α-glucosidase inhibitory activity determined by measuring the discharge of 4-nitrophenol from p-nitrophenyl α-D glucopyranoside as given by Sun et al., (1995). The assay mixtures for these experiments contained 0.3 mL of 10 Mm paranitrophenyl alpha-D- glucopyranoside, 1.0 mL of 0.1 M potassium phosphate buffer, pH - 6.8, 0.2 mL of enzyme solution and 0.2mL of varied extracts (Hexane, Chloroform, ester Methanol, and aqueous extracts) all in a final volume of 1.7mL. Following an incubation time of 30 min at 37°C, the reaction was terminated by the addition of 2.0 mL of 100mM washing soda. The liberated p-nitrophenol determined at 400 nm employing a spectrophotometer. The percentage inhibition rates were calculated using the formula. Suitable reagent blank and inhibitor controls were also carried out, and the absorbance values were subtracted.

$$\text{Inhibition (\%)} = 100\ (\text{Absorbance Control} - \text{Absorbance Test} / \text{Absorbance Control})$$

3. Results and Discussion

In the present investigation, the preliminary phytochemical screening of the crude extract *M.calabura* revealed the presence of flavonoids and polyphenols in all the extracts media viz chloroform-methanol and water with maximum yield in the aqueous extract. Flavonoids and other polyphenols are known for regulation glucose in cells of organisms in vivo. In the present study, the effects of the three extracts *M.calabura* leaves of alpha-amylase and alpha-glucosidase enzymes were studied. Diabetes management by the mechanism of antidiabetic drugs involved inhibition of both α-amylase and α-glucosidase enzymes. The inhibition of the leaf extracts *M.calabura* of the enzymes in the present study is physiologically relevant to suggest that their antidiabetic action involves the exhibition of the enzymes.

Table.1a: Qualitative Phytochemical analysis of *M.calabura* aqueous extract

S. No	Phytochemical constituents	Aqueous
1	Alkaloids	+
2	Carbohydrates	+++
3	Flavonoids	+++
4	Saponin	++
5	Steroids	++
6	Tannins	+++
7	Polyphenols	+++

+++=High, ++=moderate, +=present, −=absent

The qualitative analysis of phytochemical constituents of the aqueous leaf extracts of *Mutingia calbura* was systematically performed and recorded. The secondary metabolites such as Alkaloid, Carbohydrates, Flavonoids, Saponin, Steroids, Tannis, Polyphenols were present in the leaf extracts of *Mutingia calbura* as shown in the table (1.a).

Table.1b: Qualitative Phyto-chemical analysis of M.calabura Methanol extract.

S. No	Phyto-chemical constituents	Methanol
1	Alkaloids	++
2	Carbohydrates	++
3	Flavonoids	+
4	Saponin	+
5	Steroids	+
6	Tannins	++
7	Polyphenols	+++

+++=High, ++=moderate, +=present, −=absent

The qualitative analysis of phytochemical constituents Mutinga calbura methanol extract revealed the presence of secondary metabolites namely Alkaloid, Carbohydrates, Flavonoids, Saponin, Steroids, Tannis, Polylphenols were present in the methanol extract as shown in the table (1.b).

Table.1c: Qualitative phyto-chemical analysis of *M.calabura* Chloroform extract.

S. No	Phytochemical constituents	Chloroform
1	Alkaloids	+
2	Carbohydrates	++
3	Flavonoids	++
4	Saponin	++
5	Steroids	+
6	Tannins	++
7	Polyphenols	++

+++=High, ++=moderate, +=present, −=absent

The qualitative analysis of phytochemical constituents Mutingia calbura chloroform extract revealed the presence of secondary metabolites namely Alkaloid, Carbohydrates, Flavonoids, Saponin, Steroids, Tannis, Polyphenols were found in the chloroform extract as shown in the table (1.c).

Table.1d: Qualitative phyto-chemical analysis of *M.calabura* petroleum ether extract.

S. No	Phyto-chemical constituents	Petroleum ether
1	Alkaloids	-
2	Carbohydrates	-
3	Flavonoids	-
4	Saponin	-
5	Steroids	-
6	Tannins	-
7	Polyphenols	++

+++=High, ++=moderate, +=present, −=absent

The qualitative analysis of phytochemical constituents *Mutingia calbura* petroleum ether extract revealed the presence of secondary metabolites namely poly phenol present whereas Alkaloid, Carbohydrates, Flavonoids, Saponin, Steroids, Tannis, Polyphenols were not present in the petroleum ether extract as shown in the table (1.d).

Table.2: Alpha Amylase inhibition - *M. Calabura* Extract

Solvent	Concentration (µg/ml)				
	20	40	60	80	100
Petroleum ether	10.3	20.78	27.3	31.5	39.1
Chloroform	12.4	28.5	35.3	41.8	49.7
Methanol	18.7	32.3	40.4	54.6	74.2
Aqueous	14.7	24.4	34.9	50.8	69.9
Standard (Acarbose)	17.2	36.7	51.4	68.8	84.5

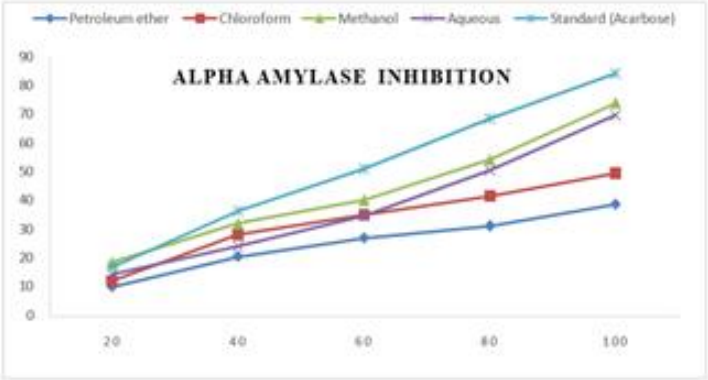

Figure.1: Shows in vitro anti-diabetic alpha amaylase activity *M. Calabura* Extract.

In vitro α-amylase inhibitory activity of leaves *(Mutingia calbura)* was studied as clarified in methods. We found that there is an increase in the ratio of inhibitory activity with the rise in dosage against-amylase. Acarbose was used as a standard drug, with related dosage to compare the inhibitory capacity of the Muntingia calbura leaves using different extraction ratios of petroleum ether to water. (Figure.11) showed that, the percentage inhibitory activity of Muntingia calbura leaves ranges a minimum of 10.3 petroleum ether (at 20µg/mL) to a maximum of 74.2 methanol extract (at 100µg/mL) whereas the Acarbose showed percentage activity ranges from 17.2 (at 20µg/mL) to a maximum of 84.5 (at 100µg/mL).

Table.3: Shows in vitro alpha-glucosidase inhibitory activity *M. Calabura* Extract.

Solvents	Concentration µg/ml				
	20	40	60	80	100
Petroleum ether	10.3	21.78	28.3	34.5	38.1
Chloroform	14.4	28.5	35.3	41.8	48.7
Methanol	21.7	32.3	40.4	49.6	66.2
Aqueous	18.7	24.4	33.9	51.8	58.9
Standard (Acarbose)	18.2	35.7	51.4	67.8	82.5

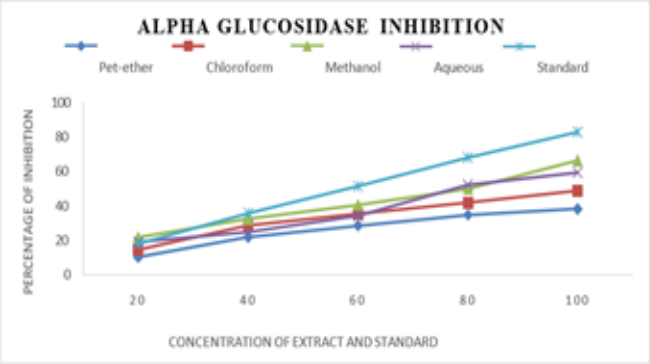

Figure.2: Shows in vitro anti-diabetic activity of alpha-glucosidase activity in *M. Calabura* Extract

Medicinal plant compounds are still the most accessible resource of α- glucosidase inhibitors. Therefore, we investigated biologically active compound from *Muttingia calabura* using different extraction ratios of petroleum ether to water. Extracts under different concentration of *Muttingia calabura* were tested for α-glucosidase inhibitory activity. Methaloic extract showed higher α-glucosidase activity than aqueous extract, whereas petroleum ether and chloroform extract did show moderate inhibit α-glucosidase at all. Especially, neat alcoholic (methanolic or ethanolic) extracts exhibited stronger inhibitory effects than their corresponding aqueous mixtures.

4. Conclusion

The crude extract of M.calabura on preliminary phytochemical screening revealed the presence of steroids, flavoinds, saponins, tannis, carbohydrate etc. The aqueous extract yielded the maximum of these compounds. The effect of beta sitosterol was investigated on the GLUT-4 expression. The result revealed the up regulation of GLUT-4 and the consequent glucose uptake in C2C12 myotube cells. Beta-sitosterol comprised 46.33 percent (area) in the above analysis. The HPLC also revealed that the presence of Stigamasterol which comprised 53.67 percent (area). The GLUT-6 Expression was enhanced by the Beta-sitosterol which suggested as a non-specific enhancement by plant extract compound. The results on both GLUT-4 and GLUT-6 are suggestive of the following points of anti - diabetic features of the M.calabura. The phyto-sterols in this plant exhibit strong stimulation of GLUT-4 transportation to the cell membranes. Both exhibit synergism or independently can elevate significantly the epigenetic expression of GLUT-4. Both can cause or bring glucose homeostasis. Both can bring the correction of the defect in the insulin mediated GLUT4 translocation and can cure both insulin dependent and insulin in dependent Diabetes mellitus. The plant M.calabura has the potential to become an effective agent in Diabetic theraphy. The above features also infer that the extract compound Beta sitosterol or similar sterol like Stigamasterol of M.calabura leaf extract may modulate at the genetic level the epigenetic expression of GLUT proteins both specifically and non-specifically.

5. References

[1] Abumweis, s. S., barake, r. And jones, p. J. (2008). Food nutr res. 52:1811.
[2] Acharya,d.and shrivastava, a. (2008) aavishkar publishers distributor, jaipur, 440.
[3] Ali, f. S. And abdel-moneim, a. A. (1989). Zentralbl.mikrobiol, 144, 623-628.
[4] Alarcon de la lastra, c.; martin, m.j.; motilva, v. (1994). Pharmacology, 48, 56-62.
[5] Antonio. C. M, munhoz, & frode, t. S. (2017). Current diabetes Reviews, 14(1).

[6] Aruna sindhe m, yadav d. Bodke and Chandrashekar A(2013) der pharmacia lettre, , 5 (3):427-435.
[7] Asuzu,i. U., & onu, o. U. (1990). International journal of crude drug research, 28(1), 27–32.
[8] Awad, a. B., & fink, c. S. (2000). The journal of nutrition, 130(9), 2127 2130.
[9] Baggio and d. J. Drucker, (2006) annual review of medicine, Vol.57.
[10] Baron ad, brechtel g, wallace p, edelman sv. (1988) am. J. Physiol. 255 e769-774.
[11] Bdelwahab, s.i., taha, m.m., abdulla, m.a., nordin, n., hadi, a.h., mohan, s., jayapalan, j.j. and hashim, o.h. (2013). J. Ethnopharmacol.148, 277–286.
[12] Bhesh raj sharma,hyun jung kim and dong young Rhyu. (2015) journal of translational medicine 13:62.
[13] Bouic pj,lamprecht jh. (1999)altern med rev. Jun;4(3):170-7.
[14] Boivin, m., flourie, b., rizza, ra., go vl and di magno, ep. (1988). Gastroenterology, 94:387–394.
[15] Brett lomenick , heping shi , jing huang , chuo chen. (2015).bioorg. Med. Chem. Lett.
[16] Changyoungyang, jingwang, yuanzhao, lishen. (2010) journal of ethnopharmacology.volume 130, 2,20 ,pages 231-236.
[17] Chnidahanswadi., junkawabata, takanorikasai. (2000)biotechnol.biochem.,64(5)1041-1043,.
[18] Chaudhury a, duvoor c, reddy dendi vs, kraleti s, chada a, ravilla r, marco a, shekhawat ns, montales mt, kuriakose k, sasapu a, beebe a, patil n, musham ck, Lohani gp, mirza w. (2017)front endocrinol (lausanne).; 8:6.
[19] Chika ifeanyi chukwuma, c. I., mopuri, r., nagiah, s., Chuturgoon, a. A., & islam, m. S. (2017). European journal of nutrition.
[20] Chipiti t, ibrahim ma, singh m, islam m. (2017). phog Mag;13suppl:323-33.
[21] Chi-ting,hsieh,t.-j.,el-shazly, m., chuang, d.-w., tsai, y.- h., yen, C.-t., chang, f.-r. (2012). Bioorganic & medicinal chemistry letters, 22(12), 3912–3915.
[22] Choudhury h, pandey m, hua ck, mun cs, jing jk, kong l, ern ly, ashraf na, kit sw, yee ts, pichika mr, gorain b, kesharwani p. (2017) j tradit complement med.;8(3):361-376.
[23] Coll, t. (2008). J. Biol. Chem. 283, 11107–11116.
[24] Cordero-herrera i, martin ma, bravo l, goya l, ramos S. (2013)mol nutr food res.;57:974–85.
[25] Croze, m., geloen, a. And soulage, c. (2015). British journal of Nutrition, 20, 14.
[26] Day, e.a. (2017). Metab. 28, 545–560
[27] Deng, y., he, k.,ye, x., chen, x., huang, j., li, x.,li, p. (2012).
[28] Journal of ethnopharmacology, 141(1), 228–233.
[29] Drucker ,cani, p. D., knauf, c., iglesias, m. A., , d. J., delzenne, n. M., & burcelin, r. (2006). Diabetes, 55(5), 1484– 1490.
[30] Dyer, j, wood, i. S., palejwala, a, ellis, a., & shirazi-
[31] Beechey, s. P. (2002). American journal of physiology-gastrointestinal and liver physiology, 282(2), g241–g248.
[32] Ebrahim-habibi, a. (2008). Drugs future, 33(suppl.a):306–307.
[33] Fediuc, s., gaidhu, m. P., & ceddia, r. B. (2005).journal of lipid Research, 47(2), 412–420.
[34] Fryer, l. G., hajduch, e., rencurel, f., salt, i. P., hundal,
[35] H. S., hardie, d. G. And carling, d. (2000). Diabetes. 49, 1978– 1985.
[36] Fujiwara, k., maekawa, f. And yada, t. (2005). American
[37] Journal of physiology-endocrinology and metabolism, 289(4), e670–e677.
[38] Fu, z., gilbert, e. R. And liu, d. (2013). Current diabetes reviews, Vol. 9, no. 1, pp. 25–53,
[39] Fu, elizabeth r. Gilbert, and dongmin liu. (2013)curr Diabetes rev. January 1; 9(1): 25–53.
[40] Gabay, o., sanchez, c. And salvat, c. (2010). Osteoarthritis Cartilage. 18:106–116.
[41] Abirami R.G and S. Kowsalya, 2013. Antidiabetic acivity of Ulva fasciata and its impact on carbohydrate metabolism enzymes in alloxan induced diabetic rats. International journal of Research in Phytochemistry and Pharmacology,3; 3:136-141.
[42] Adeyemi D.O, V.O. Ukwenya, E.M. Obuotor and S.O. Adewole, 2014. Antihepatotoxic activities of Hibiscus sabdariffa L. in animal model of Streptozotocin diabetes- induced liver damage. BMC Complement Altern Med., 30:277.
[43] Agha-Jaffar R., N. Oliver, D. Johnston and S. Robinson, 2016. Gestational diabetes mellitus does an effective prevention strategy exist?, Nature Reviews Endocrinology., 12: 533-546.
[44] Ajiboye, B.O and O.A., Ojo, 2014. Effect of aqueous leaf extract of Senecio biafrae on hyperglycaemic and haematological parameters of alloxan-induced diabetic rats. Pharmacology online, 3:163 – 169.
[45] Akhtar M.A., M. Rashid, M.I. Ibne-Wahed, M.R. Islam, S.M. Shaheen, M.A. Islam, M.S. Amran, and M. Ahmed, 2007. Comparison of long-term antihyperglycemic and hypolipidemic effects between Coccinia cordifolia (Linn.) and Catharanthus roseus (Linn.) in alloxan-induced diabetic rats. Research Journal of Medicine and Medical Sciences, 2(1):29-34.
[46] Alagammal M., A. Nishanthini and V.R. Mohan, 2012. Antihyperglycemic and Antihyperlipidaemic effect of Polygala rosmarinifolia Wright & Arn on alloxan induced diabetic rats. Journal of Applied Pharmaceutical Science, 2(9):143.
[47] Altemimi A., N. Lakhssassi., A. Baharlouei, D.G. Watson and D.A. Lightfoot, 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts, Plants, 6(4):42.

[48] American Diabetes Association Standards of Medical Care in Diabetes. Classification and diagnosis of diabetes. Diabetes Care 2016; 39 (Suppl. 1): S13-S22.

[49] Anupama V, R. Narmadha, V.K. Gopalakrishnan, and K. Devaki, 2012. Enzymatic alteration in the vital organs of streptozotocin diabetic rats treated with aqueous extract of Erythrina variegata bark. Int J Pharma Pharmaceu Sci, 4.

[50] Arima R., A. Hautakoski, M. Marttila, M. Arffman, R. Sund, P. Ilanne-Parikka, J. Kangaskokko, M. Hinkula, U. Puistola, and E. Läärä, 2017. Cause-specific mortality in endometrioid endometrial cancer patients with type 2 diabetes using metformin or other types of antidiabetic medication. Gynecologic Oncology, 147(3):678-683.

[51] Arun, G.S and K.G., Ramesh, 2002. Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocindiabetic rats. Indian J Pharmacol, 34:164.

[52] Axelsson A.S., T. Mahdi, H.A. Nenonen, T. Singh, S. Hänzelmann, A. Wendt, A. Bagge,

[53] T.M. Reinbothe, J. Millstein, X. Yang, and B. Zhang, 2017. Sox5 regulates beta-cell phenotype and is reduced in type 2 diabetes. Nature communications, 8:1-16.

[54] Azmir J., I.S.M. Zaidul, M.M. Rahman, K.M. Sharif, A. Mohamed, F. Sahena, M.H.A. Jahurul, K. Ghafoor, N.A.N. Norulaini, and A.K.M. Omar, 2013. Techniques for extraction of bioactive compounds from plant materials: a review. Journal of Food Engineering, 117(4):426-436.

[55] Balinsky, D. and R.E. Bernstein, 1963. The purification and properties of glucose-6- phosphate dehydrogenase from human erythrocyte. Biochem Biophys Acta., 67: 313-315.

[56] Benhammou N, F.A. Bekkara, T.K. and Panovak, 2008. Antioxidant and antimicrobial activities of Pistacia lentisus and Pistacia Atlanta extracts, African Journal of Pharmacy and Pharmacology, 2:22-28.

[57] Benzie, I.F.F. and J.J., Strain, 1996. Ferric reducing antioxidant power (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239: 70-76.

[58] Beutler, E., 1984. Red cell metabolism. In: A manual of biochemical methods. Grune and Starton, Inc. Orlando, FL 32887, London, 3rd Edition.

[59] Bhatia L, H. Bishnoi, P. Chauhan, K. Kinja, S. and Shailesh, 2011. In-vitro comparative antioxidant activity of ethanolic extracts of glycosmis pentaphylla and Bauhinia variegata, Recent Research in Science and Technology, 3:1–3.

[60] Blois, M.S., 1958. Antioxidant determinations by the use of stable free radical. Nature, 1: 1199-2000.

[61] Blondel, O. and B. Portha, 1992. Early appearance of in vivo insulin resistance in adult streptozotocin-injected rats. Diabetes Metab. 15, 382–387.

[62] Bonting, S.L., 2001. Sodium potassium activated adenosine triphosphatase and calcium transport membranes and ion transport. Wiley Interscience., 1:739-769.

[63] Borde J.P., N. Batin., S. Rieg., R. Feik., C. Reimling., Kern, W.V., Hübner, J., Ruhnke, M. and Kaier, K., 2014. Adherence to an antibiotic stewardship bundle targeting Staphylococcus aureus blood stream infections at a 200-bed community hospital. Infection, 42 :713-719.

[64] Bouche C, S. Serdy, C.R. Kahn and A.B. Goldfine, 2004. The cellular fate of glucose and its relevance in type 2 diabetes. Endocrine Reviews, 25: 807–830.

[65] Boukes G.J, M.V. Ventor and V. Oosthuizen, 2008. Quantitative and qualitative analysis of sterols/sterolins and hypoxoside contents of three hypoxis (African potato) spp. Afr J Biotechnol.,7:1624-9.

[66] Brandstrup, M., J.E. Kruk and C. Bruni, 1957. Determination of hexokinase in tissues. J Gerontol., 12: 166-171.

[67] Bravi M.R., A. Armiento, O. Laurenti, M. Cassano-Faldetta, O. De Luca, A.L. Morettia, 2006. Insulin decreases intracellular oxidative stress in patient with type 2 diabetes mellitus. Metabolism, 55: 591 – 596.

[68] Buege, J.A. and S.D. Aust, 1978. Microsomal lipid peroxidation in: Fleischer, S. and L. Packre, Eds. Methods in Enzymology LII, New York Academic press, 302-330.

[69] Buzzetti R., S. Zampetti and E. Maddaloni, 2017. Adult-onset autoimmune diabetes: current knowledge and implications for management. Nature Reviews Endocrinology. 1-13

[70] Carmody M., P.A, Crisp., S. Alessandro, S., Ganguly, D. Gordon., M., Havaux, M., Albrecht-Borth, V. and B.J. Pogson. 2016. Uncoupling high light responses from singlet oxygen retrograde signaling and spatial-temporal systemic acquired acclimation. Plant physiology, 171: 1734-1749.

[71] Chandramohan G, K.S. Al-Numair and K.V. Pugalendi, 2009. Effect of 3-hydroxymethyl xylitol on hepatic and renal functional markers and protein levels in streptozotocin diabetic rats. African J. Biochem Res, 3: 204.

[72] Chen J.Y., H.C. Chou, Y.H. Chen, and H.L. Chan, 2013. High glucose-induced proteome alterations in hepatocytes and its possible relevance to diabetic liver disease. The Journal of nutritional biochemistry, 24(11):1889-1910.

[73] Cordero-Herrera I. M.A. Martín, L. Bravo, L. Goya and S. Ramos, 2013. Cocoa flavonoids improve insulin signalling and modulate glucose production via AKT and AMPK in HepG2 cells.

Molecular nutrition & food research, 57(6):974-985.
[74] Cosa P., A.J. Vlietinck, D.V. Berghe, and L. Maes, 2006. Anti-infective potential of natural products: How to develop a stronger in vitro proof-of-concept. J Ethnopharmacol. 106:290-302.
[75] Daisy P, L. Vargese, and C.E. Priya, 2009. Comparative studies on the different leaf extract of Elephantopus scaber.L on STZ- induced diabetic rats. European J Sci Res, 32 (3): 304-313.
[76] Dallak, M and I. Bin-jaliah, 2010. Antioxidant activity of Citrullus colocynthis pulp extract in the RBC's of alloxan-induced diabetic rats. Pak J Physiol, 6:5.
[77] Danesh, F.R and Y.S. Kanwar, 2004. Modulatory Effects of HMG-Coa Reductase Inhibitors In Diabetic Microangiopathy, The FASEB Journal, 18: 805-815.
[78] Das S., S. Vasishat, R. Snehlata, N. Das and L.M. Srivastava, 2000. Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia. Curr. Sci., 78: 486-487.
[79] De Franco E., S.E. Flanagan., J.A., Houghton., H.L. Allen., D.J. Mackay., I.K. Temple., S. Ellard. and A.T. Hattersley. 2015. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. The Lancet, 386(9997) : 957-963.
[80] Devaki K, S. Hemmalakshmi and S. Priyanga, 2015. HPTLC analysis and in vitro antioxidant activity of aqueous bark extract of Erythrina variegata L, Israel Journal of Plant Sciences, dx.doi.org/10.1080/07929978.2015.1096608.
[81] Devi, R. and D.K. Sharma, 2004. Hypoglycemic effect of extraction of clerodendron colebrookianum walp in normal and high-fat fed rats. J. Ethanopharmacol., 90:66-68.
[82] Dilika, F., P.D, Bremner . and J.J.M, Meyer. 2000. Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites. Fitoterapia, 71(4), 450-452.
[83] Divya, B.T and S., Mini, 2011. In vitro radical scavenging activity of different extracts of
[84] Butea Monosperma bark. Int J Curr Pharm Res, 3: 114-116.
[85] Drabkin, D.L. and J.M. Austin, 1932. Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood, J. Biol.Chem., 98: 719-733.
[86] Drozdowski, L.A and A.B.R. Thompson, 2006. Intestinal sugar transport. World Journal of Gastroenterology, 12: 1657–1670.
[87] Duan X., G. Wu and Y. Jiang, 2007. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules, 12: 759–771.
[88] Duncan, B.D., 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics., 13: 359-364.
[89] Dunn, W.L., 1974. Hand book of histopathological and histochemical techniques. Redwood. Burn, Ltd., 3rd edition.
[90] Dyer J, I.S.Wood, A. Palejwala Ellis and S.P. Shirazi-Beechey, 2002. Expression of monosaccharide transporters in intestine of diabetic humans. American Journal of Physiology – Gastrointestinal and Liver Physiology , 282: 241–248.
[91] enzymes of brain and gastrochemius muscle of mice. Fluoride, 33: 17-26.
[92] Etim N.N, M.E. Williams, U. Akpabio, and E.E.A. Offiong, 2014. Haematological Parameters and Factors Affecting Their Values. Agri Sci, 2:47.
[93] Ferner RE, 1992. Drug-induced diabetes. Baillieres Clin Endocrinol Metab., 6(4):849-66.
[94] Fossati P., L. Prencipe, and G. Berti, 1980. Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clinical chemistry, 26(2):227-231.
[95] Francis G., Z. Kerem, H. P. Makkar, and K. Becker, 2002. The biological action of saponins in animal systems: a review. British journal of Nutrition, 88(06), 587-605.
[96] Friedewald W.T., R.I. Levy, and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 18(6):499-502.
[97] Gancedo, J.M. and C. Gancedo, 1971. Fructose 1,6 diphosphatase, Phosphofructokinase and glucose-6-phosphate dehydrogenase. Proceedings of the Society for Experimental Biology and Medicine., 106: 607-609.
[98] Gangwar M., M.K. Gautam, A.K. Sharma, Y.B. Tripathi, R.K. Goel and G. Nath, 2014. Antioxidant capacity and radical scavenging effect of polyphenol rich Mallotus philippenensis fruit extract on human erythrocytes: an in vitro study. The Scientific World Journal, 2014.
[99] Ganong W.F, 1999. A Review of Medical Physiology. 19th Edn., Appleton and Lange, Stanford, USA, 187-241.
[100] Gao Y., Y. Wang, X. Zhai, Y. He, R. Chen, J. Zhou, M. Li and Q. Wang, 2017. Publication trends of research on diabetes mellitus and T cells (1997–2016): A 20-year bibliometric study. PloS one, 12(9):1-13.
[101] Garratt D.C, 1964. The quantitative analysis of Drugs. Volume 3. Chapman and Hall ltd, Japan, 456-458.
[102] Garry, R. and Buethner, 1993. The pecking order of free radicals and antioxidants: Lipidperoxidation, α- tocopherol and ascorbate. Arch. Biochem. Biophys., 300: 535- 543.
[103] Gasser E., C.P. Moutos, M. Downes, and R.M. Evans, 2017. FGF1 [mdash] a new weapon to control type 2 diabetes mellitus. Nature Reviews Endocrinology.1-11.

[104] Ghadyale V., S. Takalikar, V. Haldavnekar, and A. Arvindekar, 2012. Effective control of postprandial glucose level through inhibition of intestinal alpha glucosidase by Cymbopogon martinii (Roxb.). Evidence-Based Complementary and Alternative Medicine, 2012.

[105] Ghosh S., A. Derle, M. Ahire, P. More, S. S.D. Jagtap, Phadatare, A.B. Patil, A.M. Jabgunde,

[106] G.K. Sharma, V.S. Shinde, and K. Pardesi, 2013. Phytochemical analysis and free radical scavenging activity of medicinal plants Gnidia glauca and Dioscorea bulbifera. PLoS One, 8(12):82529.

[107] Gilbert, M.P. and R.E., Pratley, 2009. Efficacy and safety of incretin-based therapies in patients with type 2 diabetes mellitus. European journal of internal medicine, 20:309- 318.

[108] Gomathi D., G, Ravikumar, M. Kalaiselvi, B. Vidya and C. Uma, 2012. HPTLC fingerprinting analysis of Evolvulus alsinoides (L.) L. Journal of Acute Medicine, 2(3):77-82.

[109]

[110] Gomathi D., G. Ravikumar, M. Kalaiselvi, B. Vidya and C. Uma, 2012. HPTLC fingerprinting analysis of Evolvulus alsinoides (L.) L. Journal of Acute Medicine, 2(3):77-82.

[111] Gomathi D., G. Ravikumar, M. Kalaiselvi, K. Devaki, C. and Uma, 2013. Effect of Evolvulus alsinoides on lipid metabolism of streptozotocin induced diabetic rats. Asian Pac J Trop Dis; 3(3): 184-188.

[112] Gonall A.G., C.S. Bardwill and M.M. David. Determinationofserum proteins by meansofbiuretreaction , BioI. Chem., 177:751-766.

[113] Gothai S., P. Ganesan, S.Y. Park, S. Fakurazi, D.K. Choi and P. Arulselvan, 2016. Natural phyto-bioactive compounds for the treatment of type 2 diabetes: inflammation as a target. Nutrients, 8(8), p.461.

[114] Goud B.J., V. Dwarakanath and B.K. Chikka swamy, 2015. Streptozotocin - A Diabetogenic Agent in Animal Models, International Journal of Pharmacy and Pharmaceutical Research. 3 (1): 253-269.

[115] Green L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok and S.R. Tannenbaum, 1982. Analysis of nitrate, nitrite and nitrate in biological fluids. Anal. Biochem., 126: 131–138.

[116] Grover J.K., V. Vats and S.S. Rathi, 2000. Anti-hyperglycaemic effect of Eugenia jambolana and Tinospora cordifolia in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol, 73: 461–70.

[117] Gruters, R.A., J.J. Neefjes., M. Tersmette., R.E. de Goede., A. Tulp., H.G. Huisman., F. Miedema. and H.L. Ploegh, 1987. Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. Nature, 330: 74.

[118] Gul M. F.Z. Kutay, S. Temocin, and O. Hanninen, 2000. Cellular and clinical

[119] Gupta, V.K and S.K. Sharma 2010. In vitro antioxidant activities of aqueous extract of Ficus Bangalensis, International Journal of Biological Chemistry, 4:134-140.

[120] Hall A.P., C.R. Elcombe, J.R. Foster, T. Harada, W. Kaufmann, A. Knippel, K. Küttler, D.E. Malarkey, R.R. Maronpot, A. Nishikawa, and T. Nolte, 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes—conclusions from the 3rd International ESTP Expert Workshop. Toxicologic pathology, 40(7):971-994.

[121] Han X., Y.L. Tao, Y.P. Deng, J.W. Yu, J. Cai, G.F. Ren, Y.N. Sun, and G.J. Jiang, 2017. Metformin ameliorates insulitis in STZ-induced diabetic mice, PeerJ, 5, p.e3155.1-13. Harborne, J.B., 1987. In: Phytochemical methods. Chapman and Hall Ltd, London. p. 354- 356.

[122] Hassan H.S, M.I. Sule A.M. Musa, K.Y. Musa, M.S. Abubakar, A.S. Hassan, 2012. Anti-Inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. African Journal of Traditional Complementary and Alternative Medicines. 9(2):250- 255.

[123] Hatami T, S.A. Emami, S.S. Miraghaee and M. Mojarrab, 2014. Total Phenolic Contents and Antioxidant Activities of Different Extracts and Fractions from the Aerial Parts of Artemisia biennis Willd, Iranian Journal of Pharmaceutical Research, 13:551-558.

[124] Hazra B, S. Biswas and N. Mandal, 2008. Antioxidant and free radical scavenging activity of

[125] Spondias pinnata, BMC Complementary and Alternative Medicine, 8:1-10.

[126] Hazra K.M, R.N. Roy, S.K. Sen and S. Laska, 2007. Isolation of antibacterial pentahydroxy flavones from the seeds of Mimusops elengi Linn. Afr. J Biotechnol. 6(12):1446- 1449.

[127] Helliwell P.A, M. Richardson, J. Affleck and G.L. Kellett, 2000. Stimulation of fructose transport across the intestinal brush-border membrane by PMA is mediated by GLUT2 and dynamically regulated by protein kinase C. Biochemical Journal, 350: 149–154.

[128] Hemmalakshmi S., S. Priyanga, and K. Devaki, 2016. Phytochemical screening and HPTLC fingerprinting analysis of ethanolic extract of Erythrina variegata L. Flowers. International journal of pharmacy and Pharmaceutical science, 8:210-217.

[129] Hemmalakshmi S., S. Priyanga. and K. Devaki. 2016. Phytochemical screening and HPTLC fingerprinting analysis of ethanolic extract of Erythrina variegata L. flowers. International Journal of Pharmacy and Pharmaceutical Sciences, 8:210-217.

[130] Horokova L, A. Licht, G. Sandig and M. Jakstadt, 2003. Standardized extracts of flavonoids increase the viability of PC12 cells treated with hydrogen peroxide: effects on oxidative injury. Arch. Toxicol. 77: 22-29.

[131] Im S.S. S.Y. Kang, S.Y. Kim, H.I. Kim, J.W. Kim K.S. Kim and Y.H. Ahn, 2005. Glucose-stimulated upregulation of GLUT2 gene is mediated by sterol response element– binding protein-1c in the hepatocytes. Diabetes, 54(6), pp.1684-1691.

[132] Ingle K.P., A.G. Deshmukh, D.A. Padole, M.S. Dudhare, M.P. Moharil, and V.C. Khelurkar, 2017. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. Journal of Pharmacognosy and Phytochemistry, 6(1), pp.32-36.Saleem, M., 2009. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer letters, 285(2), pp.109-115.

[133] International Diabetes Federation (IDF), Diabetes Atlas-8th Edition, 2017.

[134] Jackson, P. and Blythe, 2013. Immunohistochemical tecniques, In: Theory and practice of histological techniques (Eds.S.K. Suvarna, C. Layton and J.D. Bancroft), Churchill livingstone of Elsevier,Philadelphia,USA.

[135] Jan S, M.R. Khan, U. Rashid and J. Bokhari, 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of Monotheca Buxifolia fruit, Osong Public Health and Research Perspectives, 4:246-254.

[136] Jyothi K.S.N., P. Hemalathr and S. Calla, 2011. Evaluation of alpha amylase inhibitory potential of three medicinally important traditional wild food plants of India. Int J Green Pharm., 95-99.

[137] Kassirer, J.P., 1971. Clinical evaluation of kidney function: tubular function. New England Journal of Medicine, 285(9):.499-502.

[138] Kazeem M.I., J.O. Adamson and I.A. Ogunwande, 2013. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of Morinda lucida Benth leaf. BioMed research international, 2013.

[139] Kazeem, M.I. and A.O.T. Ashafa, 2015. In-vitro antioxidant and antidiabetic potentials of Dianthus basuticus Burtt Davy whole plant extracts. Journal of Herbal Medicine, 5(3):158-164.

[140] Kellet, G.L. and E. Brot-Laroche, 2005. Apical GLUT2: a major pathway of intestinal sugar absorption. Diabetes 54: 3056–3062.

[141] Kesari A.N, S. Kesari, K.S. Santosh, K.G. Rajesh and W. Geeta, 2007. Studies on the glycaemic and lipidemic effect of Murraya koenigii in experimental animals. J Ethnopharmacol, 112:305–311.