Research Article

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NATURE'S NECTAR: UNVEILING THE THERAPEUTIC POTENTIAL OF AN AYURVEDIC HERBAL BLEND IN DIABETES MANAGEMENT

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ABSTRACT

Traditional medicines derived from medicinal plants are widely used across the globe. Studying the therapeutic properties of these plants, particularly for conditions such as diabetes, is essential for developing natural treatment options. This study investigates the antidiabetic potential of an Ayurvedic herbal mixture, focusing on glucose uptake, alpha-amylase inhibition, antioxidant activity, and cytotoxicity. Various assays were employed to evaluate the herbal blend's efficacy at different concentrations. Glucose uptake was assessed using yeast cells and the HepG2 cell line, while the alpha-amylase inhibition assay measured the mixture's impact on this enzyme. The antioxidant activity was examined through a radical scavenging assay, and cytotoxicity was evaluated on HepG2 cells. Additionally, phytochemical screening identified active compounds within the herbal concoction. Results demonstrated that the Ayurvedic mixture significantly enhanced glucose uptake in yeast cells and the HepG2 cell line and inhibited alpha-amylase activity. The herbal blend also showed strong antioxidant activity, as indicated by radical scavenging. The phytochemical analysis confirmed the presence of bioactive compounds with potential therapeutic benefits, and the cytotoxicity assay revealed no significant cytotoxic effects on HepG2 cells at the tested concentrations. In summary, the study suggests that this Ayurvedic herbal mixture has promising antidiabetic and antioxidant properties, making it a potential natural therapy for diabetes. However, further research, including *in vivo* studies and clinical trials, is required to validate its therapeutic efficacy and safety.

Keywords: Diabetes Mellitus, Glucose Uptake, Antioxidants, α-Amylase, HepG2 cell line.

INTRODUCTION

Diabetes mellitus, stemming from pancreatic β-cell dysfunction, causes hyperglycemia due to insufficient insulin [1] or a combination of inadequate secretion and insulin resistance (Type2). Prolonged high blood sugar levels disrupt carbohydrate, lipid, and protein metabolism, leading to chronic complications and organ failure.[2 3]

China, India, and the US lead in diabetes prevalence, with China projected to have 140.5 million cases and India 134.2 million by 2045.[4] Globally, Non-communicable diseases account for 74% of deaths, with diabetes causing 1.6 million deaths in 2019 and are projected to reach 592 million fatalities by 2035.[1] Currently, 463 million individuals have diabetes, expected to rise to 700 million by 2045.[5]

Contemporary medications used to regulate blood glucose levels globally include insulin, thiazolidinediones (glitazone), biguanides (such as metformin), sulfonylureas, α-glucosidase inhibitors, DPP-4 inhibitors, meglitinides (glinides), and incretin mimetics. While these drugs have proven mechanisms of action, they often lead to serious side effects and adverse reactions, posing challenges in treatment. For example, metformin improves insulin sensitivity but commonly causes gastrointestinal discomfort and is contraindicated in certain conditions. Thiazolidinediones decrease insulin resistance but are associated with weight gain, fluid retention, and increased cardiovascular risk, especially in patients with liver impairment or heart failure. Rosiglitazone has been linked to heightened cardiovascular disease risk.[6]

Intensive control of postprandial hyperglycemia can mitigate diabetes-related complications, yet existing treatments often fall short. There is a need for more effective strategies, particularly in non-insulin-dependent type 2 diabetes. α-Amylase inhibition targets postprandial glucose spikes by impeding carbohydrate conversion in the small intestine.[7] Oxidative stresses, worsened by high blood sugar levels, contribute to cellular damage.[8] Plant extracts with both antioxidant and α-Amylase inhibitory properties are sought after, aiming to mitigate oxidative stress and regulate blood glucose levels effectively.

India boasts around 45,000 botanical species, thousands of which have medicinal potential. Plant-based antidiabetic therapies have been used for centuries due to their perceived safety, cost-effectiveness, and prevalence in ancient medical traditions like Indian, Korean, and Chinese practices. Some key species studied to have antidiabetic effects include *Ipomoea batatas* (Sakkarkand), *Murraya koenigii* (Curry patta), *Phaseolus*

vulgaris (Hulga), *Scoparia dulcis* (Sweet broomweed), *Vinca rosea* (Sadabahar), *Withania somnifera* (Ashwagandha).[9]

This study explores the antidiabetic activity of a formulation with medicinal plants - *Tinospora cordifolia*, *Hemidesmus indicus*, *Terminalia chebula*, and *Coriandrum sativum* using *in vitro* approaches. In traditional Indian folk medicine, *Tinospora cordifolia* regulates blood glucose by reducing oxidative stress and inhibiting gluconeogenesis, containing various beneficial compounds.[10] Additionally, *Terminalia chebula* reduces blood glucose and lipid peroxidation while enhancing glucose tolerance through its phytochemicals, akin to Acarbose's effects.[11] Indian Sarsaparilla, or *Hemidesmus indicus*, is an Ayurvedic rasayana plant with antibacterial and hypoglycaemic effects, inhibiting gluconeogenesis and reducing blood sugar levels.[12] *Coriandrum sativum* seeds possess anti-inflammatory, antioxidant, and anti-hyperglycaemic properties, offering the potential for managing both type 1 and type 2 diabetes.[13]

The integration of herbal medicine into modern medical practices faces obstacles due to insufficient clinical and scientific evidence on its efficacy and safety. Addressing this requires conducting clinical research, setting up basic bioassays for biological standardization, evaluating pharmacological and toxicological effects, and utilizing various animal models for safety assessments. Identifying active ingredients in plants is also essential. This study scientifically validates the antidiabetic activity of an aqueous herbal blend.

MATERIAL AND METHODS

Extract preparation (Antidiabetic Extract – AD Extract)
The extract was prepared using the method Tarek A. El-Desouky gave, with slight modifications.[14] Briefly, 100 mg of the herbal mixture was stirred with distilled water for hours, then filtered using a 0.22-micron filter to obtain a stock solution of 10 mg/ml.

Cell line
HepG2 cell lines were ordered from the National Center for Cell Science, Pune and kept at 37 °C at 5% CO_2 in DMEM with 10% FBS.

Phytochemical analysis for screening bioactive compounds in the AD extract
Phytochemicals were screened by methods described by Kancherla 2019.[15]

Phytochemical Fingerprinting
Samples and standards were diluted to 100 ppm concentration. The analysis utilized a C18 column with a mobile phase of methanol-water containing 1% glacial acetic acid. The mobile phase flowed at 1 mL/min at room temperature. Detection was performed at 280 nm wavelength.

Antioxidant Activity
The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay followed the Doan 2018 method.[16] AD extract concentrations were mixed with 0.004% DPPH solution and incubated in the dark. Absorbance was measured at 517 nm, with Gallic acid as the positive control. Tests were conducted in duplicates. DPPH radical-scavenging activity was calculated using a standard formula.

$$\% \text{ DPPH Radical Scavenging Activity} = [\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{AD Extract}} / \text{Absorbance}_{\text{Control}}] * 100$$

Amylase Enzyme Inhibition Assay
The α-Amylase inhibition assay followed by the method mentioned in Poovitha *et al.*[17] The AD extract was pre-incubated with α-amylase solution, then mixed with 1% starch solution, both made in sodium phosphate buffer (pH 6.9). After incubation, DNSA solution was added and boiled. Absorbance was measured at 540 nm. Distilled water served as the control, while Acarbose was the positive control. The % inhibition of α-Amylase activity was calculated.

$$\% \text{ Inhibition} = [\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{AD Extract}} / \text{Absorbance}_{\text{Control}}] * 100$$

Assessment of Glucose Absorption
A glucose absorption assay was followed by Rehman and Odeyemi's method.[18,19]

Yeast cells
Baker's yeast was soaked overnight, and a yeast suspension was prepared. Various concentrations of AD extract were combined with 15 mM glucose solution and incubated. After 10 minutes, yeast suspension was added and further incubated for 1 hour. Glucose levels were measured using a glucometer, with metronidazole as a positive control.

HepG2 cells
HepG2 cells were treated with AD extract for 48 hours, followed by incubation with media containing 15 mM Glucose. Glucose levels were measured using a Glucometer, comparing against untreated cells (negative control) and metformin (positive control). The percentage increase in glucose absorption was calculated using the given formula:

$$\% \text{ Increase in uptake of glucose} = (\text{mg/dL}_{\text{Control}} - \text{mg/dL}_{\text{Extract}}) / \text{mg/dL}_{\text{control}} * 100$$

Cytotoxicity Assay
Cytotoxicity assay was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay following Rastegar's method.[20] Cells were treated with different AD extract concentrations for 24 hours after allowing the cells to adhere. 0.2% MTT solution was added and incubated; absorbance was measured at 540 nm. Cell survival percentage was calculated relative to the positive control, paracetamol, to determine cytotoxicity.

$$\% \text{ Cell survival} = \text{Absorbance}_{\text{AD Extract}} / \text{Absorbance}_{\text{Control}} * 100$$

Statistical analysis
GraphPad Prism version 8.0 was used, and statistical analysis was performed using the One Way ANOVA and Student T-test.

RESULTS

Phytochemical Analysis
The qualitative phytochemical screening of plant extracts was performed to identify the bioactive compounds present in the extract, and the analysis revealed the presence of flavonoids, saponins, phenols and tannins, which implies their medicinal properties. Results for the same are summarized in Table 1.

Phytochemical Fingerprinting
The HPLC analysis was performed to find the specific flavonoids and tannins, such as rutin and catechol, which confirmed their presence, as demonstrated in Figures 1 A and B. The rutin and catechol concentrations were 2.554/100 ppm and 75.77/100 ppm, respectively.

Antioxidant activity
The ability of the extract to scavenge harmful radicals is calculated by its DPPH radical scavenging activity; the graph in

Figure 2 exhibited a dose-dependent increase peaking at 90.85% at a dosage of 5 mg/ml. However, at lower doses of 0.1 and 0.5 mg/ml, the extract showed no significant radical scavenging activity compared to the standard gallic acid. The performance of the extract was comparable to gallic acid, with an increase in concentration and no statistically significant difference. Pertaining to the antioxidant activity, the lowest dose of 0.1 mg/ml was not considered for further analysis.

α-Amylase Enzyme Inhibition Assay
The alpha-amylase enzyme inhibition assay was performed to examine the inhibitory activity of the extract towards alpha-amylase. The extract exhibited an 88.75% inhibition at 0.1 mg/ml, as illustrated in Figure 3, showing significant antidiabetic potential. Despite being slightly less effective than metformin, no statistically significant differences were observed between the extract and metformin control.

Assessment of Glucose Absorption
The glucose uptake assay was conducted to assess the ability of the extract to enhance glucose uptake in HepG2 cells and yeast cells. The results illustrated in Figure 4 a indicate that the AD extract consistently increased glucose uptake as its concentration increased. When compared to standard metformin, the increase in glucose uptake had a steadily growing trend. However, the difference was not found to be statistically significant. In contrast, HepG2 treated with the Ayurvedic blend showed a dose-dependent and statistically significant improvement in glucose update compared to metformin (Figure 4 b).

Cytotoxicity Assay
This assay was performed to evaluate the toxic effects of the AD extract on HepG2 cells. The results depicted in Figure 5 indicate that the extract exhibited no toxicity to the cells even at 10 mg/ml concentrations compared to paracetamol, which led to a substantial decrease in cell viability even at lower concentrations.

Table 1: Bioactive Compounds in the AD Extract

Phytochemical tests	Phytochemicals	Absent/Present
Mayer's test.	Alkaloids	Absent
Lead acetate test.	Flavonoids	Present
Salkowski's test.	Steroids	Absent
Foam test	Saponins	Present
Keller–Killiani test.	Glycosides	Absent
Ferric chloride test.	Phenols	Present
Gelatin's test.	Tannins	Present

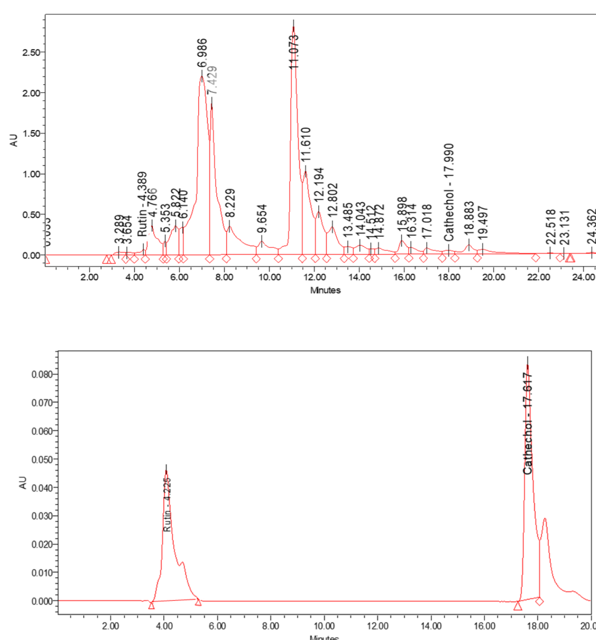

Figure 1: (a) Chromatogram of rutin and catechol, (b) chromatogram of AD extract.

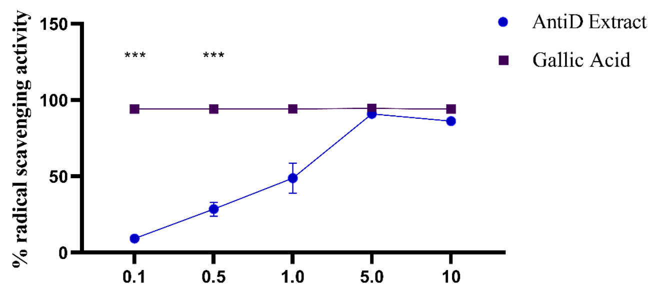

Figure 2: DPPH assay for AD Extract (p≤0.05 was considered significant when compared to standard Gallic acid)

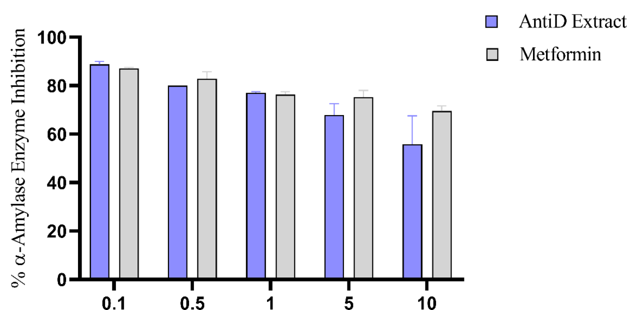

Figure 3: Amylase inhibition of AD extract compared to metformin (p≤0.05 was considered significant when compared to standard metformin)

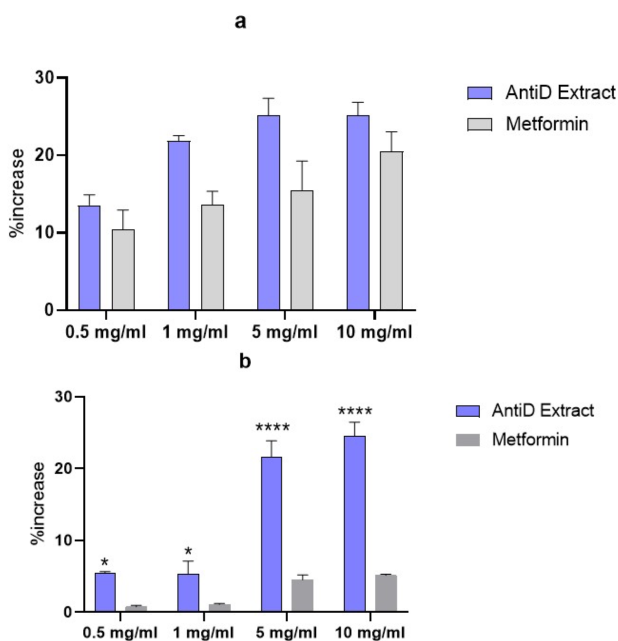

Figure 4: Percent increase in glucose uptake in (a) Yeast cells and (b) HepG2 cells (p≤0.05 was considered significant when compared to standard metformin)

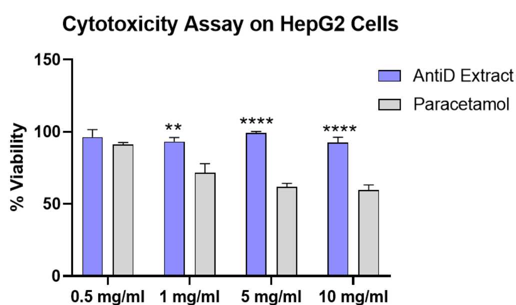

Figure 5: Cytotoxicity assay on HepG2 cells compared to paracetamol (p≤0.05 was considered significant when compared to standard paracetamol)

DISCUSSION

The study explores the antidiabetic potential of phytochemical compounds in *Tinospora cordifolia, Hemidesmus indicus, Terminalia chebula,* and *Coriandrum sativum*. These compounds, including flavonoids, saponins, phenols, and tannins, exhibit various antidiabetic effects. Catechol, a phenolic compound, enhances insulin secretion and sensitivity, inhibits glucose absorption, and modulates intracellular signalling.[21] Flavonoids act as antioxidants, mitigate oxidative stress and modulate insulin secretion and signalling. Rutin, a flavonoid, decreases carbohydrate absorption, improves tissue glucose uptake, and inhibits gluconeogenesis.[22] Saponins in *Hemidesmus indicus* increase insulin production and action, inhibit gluconeogenesis, and upregulate GLUT4 expression, enhancing glucose absorption. Furthermore, *Hemidesmus indicus's* tannin content induces glucose transport via the insulin-mediated signaling pathway.[23] The study highlights the antioxidant

potential of the AD extract by evaluating proton-donating ability and potential as a free radical suppressor. The study also revealed the efficiency of AD extract in inhibiting amylase enzymes, showing higher inhibition at lower concentrations than metformin. However, inhibition slightly decreased with increased extract concentration, possibly due to substrate saturation leading to a decline in reaction rate. This indicates the potential for managing blood glucose levels in diabetes. In type 2 diabetes, insulin resistance in peripheral tissues leads to high blood glucose levels despite pancreatic β-cell insulin production. Managing blood glucose levels, preserving tissue function, and boosting insulin sensitivity are vital.[18] The study shows that increasing concentrations of the AD extract improve glucose uptake, surpassing standard antidiabetic drugs metformin, possibly due to the presence of phenols promoting glucose uptake. Additionally, HepG2 cells were subjected to cytotoxicity evaluation using the MTT assay, which measures the breakdown of MTT salt to purple formazan crystals as an indicator of mitochondrial activity.[24] The results showed that the AD extract had high cell viability and non-toxicity of the AD extract. Its per cent viability exceeds paracetamol's, suggesting therapeutic potential and cellular health benefits.

The study identifies promising antidiabetic properties in the AD extract derived from *Tinospora cordifolia, Hemidesmus indicus, Terminalia chebula,* and *Coriandrum sativum.* These bioactive compounds demonstrate various mechanisms of action, including enhancing insulin secretion and sensitivity, inhibiting gluconeogenesis, and improving glucose uptake. Additionally, the extract exhibits antioxidant activity and low cytotoxicity, indicating its potential as a safe and effective treatment for diabetes. Further research, including *in vivo* studies and clinical trials, is warranted to validate these findings and elucidate the extract's efficacy and safety profile.

CONCLUSION

The study highlights the potential antidiabetic properties of the AD extract from *Tinospora cordifolia, Terminalia chebula, Hemidesmus indicus,* and *Coriandrum sativum*. Rich in flavonoids, saponins, phenols, and tannins, these compounds exhibit significant antidiabetic effects. Phenolic compounds like flavonoids and catechols enhance insulin secretion and cellular metabolism, while flavonoids also offer antioxidant benefits, reducing oxidative stress. The extract demonstrates potent antioxidant activity, improves glucose uptake in HepG2 and yeast cells, suppresses amylase enzyme function to regulate blood sugar levels, and maintains high cell viability without cytotoxicity. These findings underscore the AD extract's potential as a diabetic medication with diverse therapeutic mechanisms, warranting further research for clinical application.

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REFERENCES

1. Tao Z, Shi A and Zhao J. Epidemiological Perspectives of Diabetes. Cell biochemistry and biophysics 2015;73:181-185.
2. Alberti KG and Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1:diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine: A journal of the British Diabetic Association. 1998; 15: 539-553
3. Tran N, Pham B and Le L. Bioactive Compounds in Antidiabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology2020; 9: 252-283
4. Danae G *et al*. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. Lancet (London, England). 2011; 378: 31-40
5. Cho NH *et al*. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Research and Clinical Practice. 2018; 138: 271-281
6. Rynjah CV, Devi NN, Khongthaw N, Syiem D and Majaw S. Evaluation of the antidiabetic property of aqueous leaves extract of *Zanthoxylum armatum* DC. using *in vivo* and *in vitro* approaches. Journal of traditional and complementary medicine. 2018; 8: 134-140
7. Kaur N *et al*. Alpha-amylase as molecular target for treatment of Diabetes mellitus: A comprehensive review. Chemical biology & drug design. 2021; 98: 539-560
8. Bajaj S and Khan A. Antioxidants and diabetes. Indian journal of endocrinology and metabolism. 2012; 16: S267-271
9. Modak M, Dixit P, Londhe J, Ghaskadbi S and Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition. 2007; 40: 163-173
10. Saha S and Ghosh S. *Tinospora cordifolia*: One plant, many roles. Ancient science of life. 2012; 31: 151-159
11. Agrawal OD and Kulkarni YA. Treatment with *Terminalia chebula* Extract Reduces Insulin Resistance, Hyperglycemia and Improves SIRT1 Expression in Type 2 Diabetic Rats. 2023; 13: 1168
12. Gayathri M and Kannabiran K. Hypoglycemic activity of *Hemidesmus indicus* R. Br. on streptozotocin-induced diabetic rats. International journal of diabetes in developing countries. 2008; 28:6-10
13. Mechchate H *et al*. Antioxidant, Anti-Inflammatory and Antidiabetic Proprieties of LC-MS/MS Identified Polyphenols from Coriander Seeds. Molecules (Basel, Switzerland). 2021; 26: 487-500
14. El-Desouky TA. Evaluation of effectiveness aqueous extract for some leaves of wild edible plants in Egypt as anti-fungal and anti-toxigenic. Heliyon. 2021; 7: e06209
15. Kancherla N, Dhakshinamoothi A, Chitra K and Komaram RB. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of *Cayratia auriculata* (*In Vitro*). Maedica. 2019; 14: 350-356
16. Doan HV, Riyajan S, Iyara R and Chudapongse N. Antidiabetic activity, glucose uptake stimulation and α-glucosidase inhibitory effect of *Chrysophyllum cainito* L. stem bark extract. BMC complementary and alternative medicine. 2018; 18:267
17. Poovitha S and Parani M. *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC complementary and alternative medicine. 2016; 16 Suppl 1: 185
18. Rehman G *et al. In Vitro* Antidiabetic Effects and Antioxidant Potential of *Cassia nemophila* Pods. BioMed Research International. 2018: 1824790
19. Odeyemi S and Dewar J. *In Vitro* Antidiabetic Activity Affecting Glucose Uptake in HepG2 Cells following their

exposure to extracts of *Lauridia tetragona* (L.f.) R.H. Archer. 2020; 8: 33
20. Rastegar M *et al*. Investigating Effect of Rapamycin and Metformin on Angiogenesis in Hepatocellular Carcinoma Cell Line. Advanced pharmaceutical bulletin. 2018; 8: 63-68
21. Knezevic S *et al.* Catechin and other catechol-containing secondary metabolites: Bacterial biotransformation and regulation of carbohydrate metabolism. Pharma Nutrition. 2021; 17: 100273
22. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K and Büsselberg D. Flavonoids and Their Antidiabetic Effects: Cellular Mechanisms and Effects to Improve Blood Sugar Levels. Biomolecules. 2019; 9: 430-464
23. Alhujaily M, Dhifi W and Mnif W. An Overview of the Potential of Medicinal Plants Used in the Development of Nutraceuticals for the Management of Diabetes Mellitus: Proposed Biological Mechanisms. 2022; 10: 2044
24. Ghasemi M, Turnbull T, Sebastian S and Kempson I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. International Journal of Molecular Sciences. 2021; 22:12827-12857

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