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Formulation and phytochemicals characterization of polyherbal (*Tinospora cordifolia*, *Gymnema sylvestre*, *Pterocarpus marsupium* and *Acacia arabica*) antidiabetic compressed tablet lozenges

Suman Yadav, Pratibha Nand, Rajinder K. Gupta

Abstract
The goal of the study was to supply slow release medicament for the treatment of diabetes by formulating polyherbal antidiabetic compressed tablet lozenges. There is still a demand for new dosage forms that act effectively despite various other forms of dosages such as powder, tablets and injectable being available in the market. So the lozenges are the new and innovative way for drug delivery to provide a synergistic effect. Increased retention time of dosage form in the oral cavity is the major advantage of the medicated lozenges. This leads to an increased bioavailability and reduction in gastric irritation and bypass metabolism. The lozenges were prepared by a wet granulation method using *Tinospora cordifolia, Gymnema sylvestre, Pterocarpus marsupium* and *Acacia arabica* herbal extracts and other excipients like sorbitol, mannitol, PEG 6000 with lemon flavour and menthol. PVP was used as a binder, while Stevia powder was used as a sweetener. These herbs are widely used as sources of bioactive compounds like phytochemicals, antioxidants as well as for their antidiabetic effects. The formulated lozenge was subjected to various physicochemical parameters like hardness, thickness, and friability and disintegration time. The hardness of lozenges was found to be 20.3 Kg/cm². Friability was 0.59%. Thickness of lozenges was found to be 5.0 mm and disintegration time was 17 minutes. Upon carrying out of a stability study at 30 ºC & 40 °C for a period of one month, it was observed that there is no change in physico-chemical properties. The compressed tablet lozenges can provide an effective substitute for the treatment of diabetes. HPLC was carried out to identify bioactive compounds like quercetin (0.5062%), kaempferol (0.0864%), epicatechin (0.0191%) and rutin (1.2726%) which are known for their antidiabetic and antioxidant effect. The presence of Phytol, 1H-Indene, 1-ethylideneoctahydro-7 a-methyl-, (1E,3a.alpha.,7a.beta.), 2H-1-Benzopyran,6,7-dimethoxy-2,2-dimethyl, Inositol,1-deoxy, 2-Methoxy-4-vinylphenol, 2-methoxy-3-2-propenylphenol-, 2-Ethylacridine, delta-selinene and fatty acids were identified by the GC-MS analysis of various hydro alcoholic herbal extracts. All of these have significant therapeutic implications.

Keywords: Polyherbal antidiabetic compressed tablet lozenges, wet granulation method, HPLC, GC-MS, *Tinospora cordifolia, Gymnema sylvestre, Pterocarpus marsupium* and *Acacia arabica* extracts.

Introduction
Diabetes mellitus is a world's largest endocrine disease characterized by hyperglycemia, hyperlipedemia, hypoinsulinaemia and hyperaminoacidemia [1]. Insulin and various oral antidiabetic agents such as glinides, α-glucosidase inhibitors, sulfonylureas and biguanides, are some of the currently available therapies for diabetes [2]. Products are not easily accessible as well as expensive in developing countries. In the contemporary world, diabetes mellitus is becoming increasingly problematic. India has today become the diabetic capital of the world with over 20 million diabetic patients. According to estimates, this figure is likely to escalate to 57 million by 2025 [3]. Hypoglycaemia, flatulence, nausea, alcohol flush, vomiting, headache, hyponatremia, weight gain, diarrhoea or constipation, joint pain, lactic acidosis, dizziness, pernicious anaemia, dyspepsia are some of the side effects of allopathic drugs used for treatment of diabetes. Hence, herbal drugs are a great substitute for allopathic drugs as they have little to no adverse effects [4]. About 70% of the world's population use traditional medicines derived from medicinal plants. In the present work, we had developed lozenges, using *Tinospora cordifolia, Gymnema sylvestre, Pterocarpus marsupium* and *Acacia arabica* having proven record of antidiabetic effects.
Tinospora cordifolia is commonly known as giloy or guduchi, belongs to the family Menispermaceae. The alkaloids such as magnoflorine, jatrorrhizine, palmatine present in the *T. cordifolia* and tannins, flavonoids, glycosides, saponins, steroids aid in curing diabetes [5].

Another herb, *Gymnema sylvestre* (gudmar) belongs to the family Apocynaceae contains gymnemic acids (anti sweet compounds) in the leaves of gudmar. It founds its application in maintaining the levels of insulin, lowers blood sugar level and suppresses the transport of glucose molecule from intestine into the blood stream [6, 7]. Antisweet constituent of the leaves has been found to be a mixture of triterpene saponins. Sensitivity to sweet substances declines as a result of chewing of these leaves [8]. *Pterocarpus marsupium* also known as Indian Kino tree and in Hindi it is known as vijaysar, belongs to the family, Fabaceae. Pterostibene, marsupin significantly lowers the blood glucose level [9]. (-)-Epicatechin has been found to be insulinogenic and it also leads the conversion of proinsulin to insulin [10]. *Acacia arabica* is commonly known as babool and is a bark of a tree that belongs to family Leguminosae. Administration of powdered seeds of *Acacia arabica* (2, 3 and 4 g/kg body weight) was very effective by the production of insulin from pancreatic beta cells [11].

Though herbal drugs are used in various Ayurvedic preparations such as a powder, tablet, syrup, decoction to treat diabetes, but a new way of drug delivery called the lozenge form is advantageous over the existing formulations. Lozenges are the flavoured medicated dosage forms intended to be sucked and held in the mouth or pharynx containing one or more medicaments. Lozenges increase the retention time of the dosage form in oral cavity leading to increase bioavailability of active compounds, reduction in gastric irritation and bypass metabolism process [12, 13].

The aim of the present study is a formulation and phytochemicals characterization of polyherbal (*Tinospora cordifolia, Gymnema sylvestre, Pterocarpus marsupium* and *Acacia arabica*) antidiabetic compressed tablet lozenges.

2. Materials and Methods

2.1. Herbal extracts used

Tinospora cordifolia, Gymnema sylvestre, Pterocarpus marsupium and *Acacia arabica* herbal extracts were supplied by kisalaya herbals limited, Indore. The herbal extracts used were labelled as *Tinospora cordifolia* (TC), *Gymnema sylvestre* (GS), *Pterocarpus marsupium* (PM), *Acacia arabica* (AA) and mixture of these herbal extracts (MIX).

2.2. Chemicals

Acetic acid, aluminium chloride, ascorbic acid, catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride, Folin Ciocalteu's phenol reagent, gallic acid, sodium acetate, sodium carbonate, sodium nitrite, 2,4,6-Tripyridyl-s-triazine (TPTZ), hydrochloric acid, n-butanol, sodium hydroxide, tannic acid, potassium ferrocyanide, methanol, ethanol, diethyl ether, BHT, DMSO, potassium di-hydrogen orthophosphate, milli Q water, acetonitrile, hydrochloric acid, benzene, chloroform, ammonium hydroxide, potassium hydroxide, nutrient agar, nutrient broth, conc. H_2SO_4 were procured from food processing laboratory, USBT, Guru Gobind Singh Indraprastha University, New Delhi. All chemicals were of analytical grade. Other materials used for antidiabetic herbal lozenges are sorbitol, mannitol, citric acid, stevia, PVP, natural menthol, magnesium stearate, silica, PEG 6000, talc, flavour (lemon).

2.3. Bacterial strains

Eschericha coli, Bacillus subtilis, Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermidis and *Salmonella enterica* were supplied by microbiology laboratory, USBT, Guru Gobind Singh Indraprastha University, New Delhi.

2.4. Qualitative phytochemical analysis

The hydro alcoholic herbal extracts of TC, GS, PM, AA and MIX were subjected to different phytochemical tests such as terpenoids, phytosterol, coumarin, anthraquinones, phlobatannins. The presence of terpenoids were determined by the method described by Harborne [14]. The coumarins present in all the hydro alcoholic herbal extracts were detected by the method described by Gopinath *et al* [15]. Salkowski test was used for the detection of phytosterols [14]. Phlobatannins were detected by the method given by Edeoga *et al* [16]. Anthraquinones present in all the herbal extracts were determined by the method described by Trease and Evans [17].

2.5. Quantitative phytochemical analysis

The total phenolics were estimated using the Folin-Ciocalteu assay [18]. The absorbance was measured at 765 nm and the results were expressed in terms of gallic acid equivalents. The total flavonoids were analyzed using Aluminiun Chloride colorimetric method [19]. Absorbance was measured at 510 nm. Results were expressed in terms of catechin equivalents.

Tannins were analyzed according to the method described by Van-Buren and Robinson [20], with some modification. Absorbance was measured at 605 nm within 10 minutes and the results were expressed in tannic acid equivalents. The saponins content was determined according to the procedure described by Obadoni and Ochuko [21]. The saponin content was calculated as percentage:

$$\text{Saponin \%} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100$$

2.6. Antioxidant capacity evaluation

2.6.1. DPPH radical scavenging activity

DPPH radical scavenging activity was analyzed by the method of Blois, 2000 [22] with slight modification. A 10 mg (1000PPM) of extract was dissolved in 10 mL methanol and then 1mL of sample was taken from 10 mL and dissolved with 1mL of 0.3mM methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl), 1 mL methanol in a test tube. After this, test tube was incubated in the dark for 10 minutes. Blank was prepared using 2ml methanol and 1ml DPPH. Methanol was used as a reference. Then absorbance was taken at 517 nm.

A radical scavenging activity was expressed by % of scavenging activity and was calculated by the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD Blank} - \text{OD Sample}}{\text{OD Blank}} \times 100$$

2.6.2. Ferric reducing antioxidant power (FRAP)

The FRAP assay was determined according to the procedure of Benzie and Strain [23]. The FRAP reagent was freshly prepared by mixing 2.5 mL of TPTZ (10 mM in 40 mM hydrochloric

acid), 2.5 mL of ferric chloride (20 mM) and 25 mL of sodium acetate buffer (300 mM, pH 3.6). A 100 µl of extract was mixed with 900 µl of FRAP reagent. The mixture was allowed to stand for 4 minutes at 37 °C. The absorbance was taken at 593 nm and the result was expressed as BHT equivalents.

2.7. GC-MS Profiling

The Gas chromatography-Mass spectrometry (GC-MS) analysis was carried out for the hydro alcoholic herbal extracts of antidiabetic herbs. The carrier gas used was Helium. An Agilent 6890 GC with 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for the sample. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library [24]. The temperature for the analysis was maintained at 300 °C, solvent delay was 3 min, ion source and quadruple temperature were 230 °C and 150 °C, respectively [25]. The detected compounds have been identified by NIST'05 mass spectrum library. The identity of the secondary metabolites in the hydro alcoholic herbal extracts was carried out by mass spectroscopy based on the comparison of their retention time.

2.8. HPLC analysis of herbal mixture extract

A simple, specific, accurate and precise high performance liquid chromatography method was developed for the simultaneous estimation of quercetin and kaempferol and also for epicatechin and rutin in a mixture of herbal extract (MIX).

2.8.1. HPLC instrumentation

Agilent infinity 1200 series system was used for the quantification of bioactive compounds which comprising a pump, an automatic sampler and a diode array (DAD) detector was used with data acquisition by EZ chrom elite software.

2.8.2. Preparation of reference and test solution

A stock solution of reference standards like quercetin, kaempferol, epicatechin and rutin were prepared. For this accurately weighed amount of quercetin, kaempferol, epicatechin and rutin standard (1mg) were dissolved in 5 ml of methanol separately, in a volumetric flask. For test solution, 50 mg sample (mixture of herbal extracts) was dissolved in 5 ml of methanol. Reference and test solutions were then sonicated for 5-10 minutes and then the contents of the flask were filtered.

2.8.3. Chromatographic system for reference and test solution

The HPLC conditions for quantification of quercetin and kaempferol were developed by the Indian Pharmacopoeia [26] and for epicatechin and rutin, the conditions were developed by Nour *et al* [27].

- Stationary phase used for the quercetin, kaempferol, epicatechin and rutin was a stainless steel column (25cm x 4.6mm) packed with octadecylsilane bonded to porous silica (5µm).
- Mobile phase used for the quercetin and kaempferol was a mixture of 40 volumes of acetonitrile and 60 volumes of buffer solution, prepared by dissolving 3.01g of potassium di-hydrogen orthophosphate in 1000 ml of milli Q water and the pH 2.0 with acetic acid was adjusted. For epicatechin and rutin, the mobile phase consists of solvent A (1% acetic acid) and solvent B (methanol).
- Flow rate-1 ml/minute.
- For quercetin and kaempferol, Spectrophotometer set at 370 nm and for epicatechin and rutin, wavelength was 278 nm.
- Injection volume-10µl.
- Run time-15 minutes.
- Column temperature-25 °C

Reference and test solution were injected.

The percent of quercetin, kaempferol, epicatechin and rutin present in the herbal mixture extract was calculated by using a formula:-

$$(\%) = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard (mg)}}{\text{Standard dil.}} \times \frac{\text{Sample dil.}}{\text{Sample (mg)}} \times \frac{\text{Standard potency}}{100}$$

Where dil= dilution.

2.9. Antibacterial activity

Agar well diffusion method was used for antibacterial activities of the herbal mixture extract against four Gram-positive bacteria *(Bacillus subtilis, Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermidis*) and two Gram negative bacterial (*E. coli, Salmonella enterica*) test pathogens [28]. The final concentrations of herbal mixture extract were 50 and 100 mg/ml. Nutrient agar was inoculated by spreading 100µl of the bacterial inoculums. Wells (6 mm diameter) were punched in the agar and 100µl of the herbal mixture extract was loaded into the wells and then for 24 hours, the plates were incubated at 37 ºC. After 24 hours of incubation, the diameter of the zone of inhibition (mm) was measured. DMSO and methanol were used as control.

2.10. Method for compressed tablet Lozenges formulation

Compressed tablet lozenges were prepared by the wet granulation method. Accurately weighed amount of sorbitol, mannitol, herbal extracts (TC, GS, PM, AA), citric acid and stevia were mixed thoroughly by trituration and was granulated using the wet granulation method using PVP solution as a binder. Wet mass was then passed through sieve #22 and the granules obtained were dried at 40 °C for 4-5 hours. Dried granules then passed through sieve #44 and 10% fines were also added. Remaining magnesium stearate, silica, PEG 6000, talc, flavour and natural menthol were then added to dried granules, mixed and subjected to compression using a rotary punching machine. Each tablet lozenge weighed approximately 1.0 gm [29].

Table 1: Composition of polyherbal antidiabetic compressed tablet lozenges.

Ingredient	Quantity (%)
Sorbitol	43.497
Mannitol	39.758
Herbal extracts	7.998
Citric acid	0.4299
Stevia	0.8997
PVP	0.0144
Menthol	0.0319
Magnesium stearate	2.691
Silica	1.357
PEG 6000	0.969
Talc	0.213
Flavor	2.142
Water Q.S.	13.15

Where, QS= Quantity sufficient.

2.11. Evaluation and characterization of compressed tablet lozenges

The prepared polyherbal antidiabetic compressed tablet lozenges were studied for their physicochemical properties like moisture, hardness, thickness, friability, disintegration time, stability and organoleptic parameters (colour, taste, odour, and texture).

2.11.1. Moisture analysis

For Karl Fischer titration, 20ml of dehydrated methanol was added to the titration vessel and was titrated to the electrometric end point using Karl Fischer reagent. Prescribed amount of substance was weighed accurately and quickly transferred to the titration vessel. Stirred for one minute and titrated again to the electrometric end point using KF reagent. Now, lozenges were crushed in pestle mortar, weighed 4-5 times and then added in the KF reagent to attain a moisture percent value [30].

2.11.2. Lozenge hardness

The Pfizer hardness tester was used to measure the lozenges hardness in terms of kg/cm^2. Hardness of lozenge is the measurement of force which is applied across the diameter of the lozenge in order to break it as chipping or breakage during storage and handling always depends on the hardness [31].

2.11.3. Lozenge thickness

The thickness of lozenges was measured by vernier calliper and it is a significant feature in reproducing appearance. The average thickness for lozenges was measured in mm and presented with standard deviation [31].

2.11.4. Friability

It is a measure of mechanical strength of tablets and was determined using Roche Fribilator. Ten lozenges were pre-weighed and then they were placed in the Friabilator. The lozenges were then rotated at 25 rpm for 4 minutes (100 rotations) and then the lozenges were re-weighed [31]. Loss in the weight of lozenges is the measure of friability and is expressed as:

$$\% \text{ Friability} = [(W1 - W2) / W1] \times 100$$

Where W1 = Initial weight of 10 lozenges

W2 = Weight of the 10 lozenges after testing

2.11.5. Disintegration time

Disintegration time is defined as the time interval required for complete disappearance of a tablet or its particles from the tester net. Disintegration test apparatus was used to determine the disintegration time using phosphate buffer, pH 6.8 at 37 ºC [32].

2.11.6. Stability studies

The stability studies were conducted at 30 °C and 40 °C over a period of one month on the compressed tablet lozenges. Amber coloured bottles were used in which 10 lozenges were kept which was then maintained at 30 °C & 40 °C. Samples were then analyzed at an intervals of 7, 15 and 30 days for the physicochemical properties [29].

2.11.7. Sensory evaluation of compressed tablet lozenges.

The sensory evaluation was carried out to assess the overall acceptability of the polyherbal antidiabetic compressed tablet lozenges. The sample was evaluated for colour, taste, odour and texture. Compressed tablet lozenges were then analyzed for overall liking of the sample by 10 members using a nine-point hedonic scale.

3. Results and Discussion

3.1. Qualitative and quantitative phytochemical analysis

Hydro alcoholic herbal extracts (TC, GS, PM, AA and MIX) were subjected to phytochemical analysis, both for qualitative and quantitative. Qualitative tests showed the positive results for phytosterol and terpenoids (Table 2). Phytosterols are very useful in decreasing the levels of cholesterol and the consumption of phytosterols reduces coronary heart disease. It is also useful for the inhibition of cancer growth [33]. Isoprenoids are also known as terpenoids (terpenes) and they are the largest group of phytochemicals and used as a therapeutic and chemopreventive agents in liver cancer. It also possesses an antioxidant activity [34].

Table 2: Qualitative analysis of various hydro alcoholic herbal extracts to screen for the presence of phytochemicals.

Herbalextracts	Phytosterol	Terpenoids	Phlobatannins	Anthraquinone	Coumarin
TC	+	+	-	-	-
GS	+	+	-	-	-
PM	+	+	-	-	-
AA	+	+	-	-	-
MIX	+	+	-	-	-

Where, + is present and - is not present.

Phytochemicals originate from the plant and they are bioactive chemicals. All the bioactive compounds are synthesized naturally in any part of the plant such as stem, leaves, bark, flower, seeds, root, fruits etc. [35]. Phytochemicals of herbal extracts may have valuable effects on alzheimer's disease, diabetes, cancer, heart disease, high blood pressure and also reduce post-prandial blood glucose levels. Phenolic compounds, tannins, flavonoids and alkaloids are the most significant bioactive compounds of plants and herbal extracts are the rich source of polyphenolic compounds. Phenolic compounds are antioxidants and secondary metabolites and they contribute to nutraceutical value such as antimicrobial effect, modulation of detoxification enzymes, modulation of hormone metabolism, stimulation of the immune system, decrease of platelet aggregation and anticancer property [36].

Flavonoids are very important and diverse group of phytochemicals and also act as antioxidants. Hydro alcoholic herbal extracts showed the phenolic compound, flavonoids, tannins and saponins in a significant amount (Table 3).

Table 3: Total phenol, total flavonoid, tannin and saponin content of various herbal hydro alcoholic extracts.

Herbal extracts	Total phenolic content (µg GAE/ mg sample)	Total flavonoid content (µg CE/mg sample)	Tannin content (µg TAE/mg sample)	Saponin (%)
TC	3.12	36.80	0.1335	4
GS	57.25	62.40	0.101	8
PM	62.63	35.20	0.0835	7.5
AA	204	306	0.031	3.4
MIX	45.31	99.20	0.101	7

3.2. Determination of antioxidant activity

Free radicals are harmful as they are responsible for oxidative stress. They react with cellular components such as DNA, proteins and may lead to cell damage [37]. Large amount of free radicals have been associated with some of the chronic diseases of liver, heart and cancers. Free radicals lead to degenerative diseases and they are involved in the pathogenesis of cardiovascular disorders, cancer, diabetes, inflammation, nephrotoxicity, liver damage, the process of aging and in the neurological disorders. Antioxidant phytochemicals in herbal extracts plays an important role in preventing and treating many human diseases [38] and these antioxidants from the human body, scavenge the free radicals and decreasing the risk of degenerative diseases which is associated with aging [39].

3.2.1. DPPH radical scavenging activity

This is the most widely reported method for determining the antioxidant activity of many plant drugs. DPPH is a stable free radical with violet colour. If free radicals have been scavenged, DPPH will change its colour from violet to pale yellow or colourless. This property allows visual monitoring at 517 nm. A scavenging activity in % inhibition of hydro alcoholic herbal extracts is given in Table 4. Antioxidants prevent the cells against the harmful effects of reactive oxygen species, such as super oxide, hydroxyl radicals, singlet oxygen, peroxyl radicals and peroxynitrite.

The result revealed that the hydro alcoholic extract of *Gymnema sylvestre* exhibited the highest antioxidant activity by DPPH assay. The percent inhibition was found to be 83.99% at 1000PPM. This is due to the higher content of phenols, flavonoids, saponins, alkaloids, glycosides, tannins etc. present in the leaves of *Gymnema sylvestre* indicating presence of natural antioxidants [40]. The antioxidant activity of *Pterocarpus marsupium* heart wood was less than *Acacia arabica*. The antioxidant activity of *A. arabica* may be due to the hydroxyl groups, present in the phenolic compounds which are responsible for scavenging the free radicals [41].

Table 4: DPPH assay of various hydro alcoholic herbal extracts.

Herbal extracts	% Inhibition
TC	45.80
GS	83.99
PM	75.14
AA	83.20
MIX	61.72

3.2.2. Ferric reducing antioxidant power (FRAP)

This method is based on the principle of reduction of ferric tripyridyl-s-triazine complex to ferrous coloured form in the presence of antioxidants. The procedure was described by Benzie and Strain [23]. The antioxidants present in the herbal extracts reduces ferric tripyridyl-s-triazine complex to form a blue coloured complex which results in an increase in the absorbance at 593 nm. The BHT equivalent to the respective herbal extracts is mentioned below (Table 5). The result of the present study indicated that TC, GS, PM, AA and MIX extracts have good antioxidant activity with high levels of flavonoids and polyphenols.

Table 5: BHT equivalents (µg BE/mg sample) for various herbal hydro alcoholic extracts.

Herbal extracts	BE equivalents (µg BE/ mg sample)
TC	394
GS	806
PM	796.8
AA	7785
MIX	1806

3.3. Characterization of GC-MS analysis

The GC-MS analysis of hydroalcoholic herbal extracts resulted in identification of phytochemicals and common fatty acid. The results obtained indicated that the main compounds were Phytol, 1H-Indene, 1-ethylideneoctahydro-7 a-methyl-, (1E,3a.alpha.,7a.beta.), 2H-1-Benzopyran,6,7-dimethoxy-2,2-dimethyl, Inositol,1-deoxy, 2-Methoxy-4-vinylphenol, 2-methoxy-3-2-propenylphenol-, 2-Ethylacridine, delta-selinene hexadecanoic acid, 9, 12-Octadecadienoic acid (Z,Z)- etc. (Table 6-10).

Phytol (3, 7, 11, 15-tetramethylhexadec-2-en-1-ol) is a diterpene. The terpene exhibited an antioxidant activity and

antinociceptive properties that include the citronellal [42], monoterpenes carvacrol [43] as well as the rographolide and diterpene [44]. Phytol is the product of chlorophyll metabolism in plants. Phytol performs so many functions in the human body due to manufacturing of vitamin K and E. Phytol is associated with the enzymes which are responsible for insulin production and it is also important in lowering down the levels of blood cholesterol [45]. Phytol is known to prevent the teratogenic effects of retinol [46] and also known to inhibit the growth of *Staphylococcus aureus* [47]. Phytol possess a strong antioxidant activity to stop the development of thiobarbituric acid reactive substances (TBARS) and also eliminate nitric oxide and hydroxyl radicals. It also shows antinociceptive effects both through its central and peripheral actions [48].

Palmitic acid known as hexadecanoic acid and possesses anti-atherosclerotic and mild antioxidant properties [49]. Pentadecanoic acid, 14-methyl-, methyl ester exhibited an antioxidant effect [50]. 9, 12-Octadecadienoic acid (Z, Z)-(linoleic acid) is very useful as antiacne, hypocholesterolemic, hepatoprotective, anticoronary, anti- androgenic, antihistaminic and many more [51]. Another compound was 2-Methoxy-4-vinylphenol which showed the strong anti-inflammatory activity through the elimination of NF-κB and MAPK activation. It is a phenolic compound and used as a flavouring agent [52]. 2-Methoxy-4-vinylphenol can arrest abnormal progression of cell cycle by preventing the hyper-phosphorylation of retinoblastoma protein in chemical carcinogen-treated NIH 3T3 cells [53].

Table 6: Secondary metabolites in GC-MS analysis of *Tinospora cordifolia* (Hydro alcoholic extract).

Compound Name	CAS#	RT	% Area
Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	006876-13-7	18.957	0.52
Hexadecanoic acid, methyl ester	000112-39-0	19.865	0.26
n-Hexadecanoic acid	000057-10-3	20.302	1.90
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	21.973	3.68

Table 7: Secondary metabolites in GC-MS analysis of *Gymnema sylvestre* (Hydro alcoholic extract).

Compound Name	CAS#	RT	% Area
2-Methoxy-4-vinylphenol	007786-61-0	12.642	0.90
Phenol, 2-methoxy-3-(2-propenyl)-	001941-12-4	13.248	0.46
2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	000644-06-4	17.106	13.00
Inositol, 1-deoxy-	062076-18-0	18.564	5.51
Hexadecanoic acid, methyl ester	000112-39-0	19.820	0.60
n-Hexadecanoic acid	000057-10-3	20.280	3.02
Heptadecanoic acid	000506-12-7	21.188	0.58
Phytol	000150-86-7	21.659	1.51
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	21.951	4.66
2-Ethylacridine	055751-83-2	28.187	1.75

Table 8: Secondary metabolites in GC-MS analysis of *Pterocarpus marsupium* (Hydro alcoholic extract).

Compound Name	CAS#	RT	% Area
delta.-Selinene	028624-23-9	16.871	0.44
1H-Indene, 1-ethylideneoctahydro-7 a-methyl-, (1E,3a.alpha.,7a.beta.)	056324-68-6	17.140	0.57
n-Hexadecanoic acid	000057-10-3	20.314	1.83
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	21.985	1.37

Table 9: Secondary metabolites in GC-MS analysis of *Acacia arabica* (Hydro alcoholic extract).

Compound Name	CAS#	RT	% Area
Hexadecanoic acid, methyl ester	000112-39-0	19.887	0.58
n-Hexadecanoic acid	000057-10-3	20.347	1.86

Table 10: Secondary metabolites in GC-MS analysis of herbal mixture extract (Hydro alcoholic extract).

Compound Name	CAS#	RT	% Area
2-Methoxy-4-vinylphenol	007786-61-0	12.687	0.88
Pentadecanoic acid, 14-methyl-, methyl ester	005129-60-2	19.831	0.96
n-Hexadecanoic acid	000057-10-3	20.258	5.28
Phytol	000150-86-7	21.659	1.43
9-Octadecenoic acid, (E)-	000112-79-8	21.929	6.07

3.4. HPLC analysis of herbal mixture extract

HPLC is one of the best separation techniques of column chromatography for the quantification of different phytochemicals. In the present study, four of the phytochemicals such as quercetin, kaempferol, epicatechin and rutin were analyzed by using HPLC method. HPLC method was developed for the quantitative estimation of quercetin, kaempferol, epicatechin and rutin from hydro alcoholic herbal mixture extract with a stainless steel column (25cm x 4.6mm) which was packed with octadecylsilane bonded to porous silica (5µm) as a stationary phase.

HPLC spectra of quercetin and kaempferol present in the herbal mixture extract shows the peak at retention time of 5.393 and 8.153 minutes respectively which is shown in Fig. 1. People who are suffering with diabetes develop so many problems such as neuropathy, retinopathy, diabetic cataracts, and nephropathy in the body. So, flavonol like quercetin may be useful in the nutritional management of diabetes [54]. The best properties of quercetin, kaempferol and its glycosides are that they acts as antioxidants and antidiabetic [55].

Epicatechin and rutin showed the peak at retention time of 4.193 and 6.093 minutes respectively which is represented in Fig. 2. Epicatechin and rutin were known for their antidiabetic

properties and also act as an antioxidant. According to Ahamad *et al*, (-)-Epicatechin raised the c-AMP (cyclic adenosine monophosphate) content of the islets which is associated with increased insulin production. They observed that the (-)-epicatechin was responsible for the conversion of proinsulin to insulin [56].

Percentage of quercetin, kaempferol, epicatechin and rutin present in the herbal mixture extract was calculated using a formula:-

$$(\%) = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard (mg)}}{\text{Standard dil.}} \times \frac{\text{Sample dil.}}{\text{Sample (mg)}} \times \frac{\text{Standard potency}}{100}$$

Where dil = dilution.

The concentration of quercetin, kaempferol, epicatechin and rutin present in herbal mixture extract is given in Table 11.

Table 11: HPLC analysis of quercetin, kaempferol, epicatechin and rutin in the herbal mixture extract.

Compound	Retention time	Area	Concentration of compound in herbal mixture extract (%)
Quercetin	5.393	40860	0.5062
Kaempferol	8.153	3340	0.0864
Epicatechin	4.193	798892	0.0191
Rutin	6.093	39614	1.2726

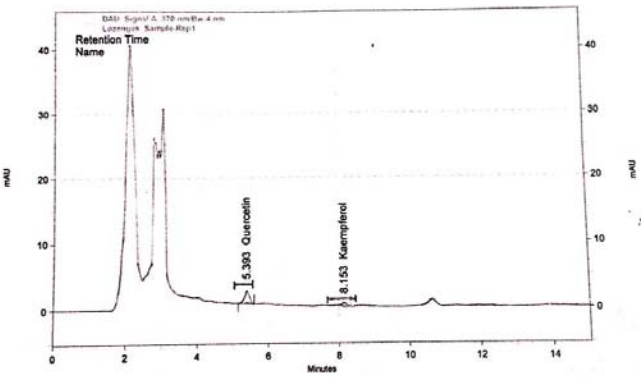

Fig 1: Estimation of quercetin and kaempferol in herbal mixture extract.

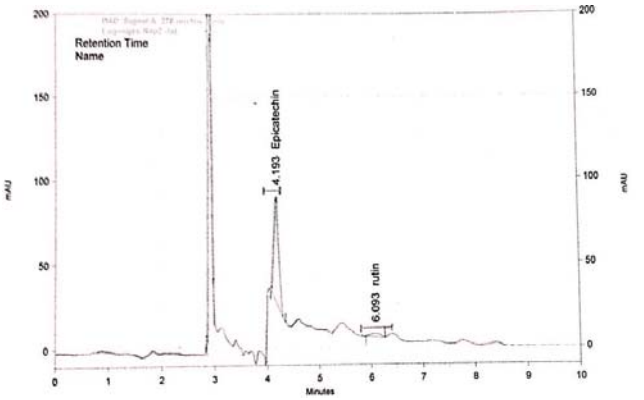

Figure 2: Estimation of Epicatechin and rutin in herbal mixture extract.

3.5. Antimicrobial activity

Four gram positive *(Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis*) and two gram negative (*E. coli, Salmonella enterica*) bacterial test pathogens were used to evaluate the antibacterial activity of herbal mixture of four extracts (TC, GS, PM and AA). The method used was agar well diffusion to assess the activity against the bacterial test pathogens. Results were analyzed by measuring the zones of inhibition using 50 mg/ml and 100 mg/ml concentrations of herbal mixture extract. Antibacterial activity was analyzed against the DMSO and methanol control. The mixture of hydro alcoholic herbal extract showed significant zones of inhibition (mm) for the bacterial test pathogens given in Table 12.

Table 12: Antibacterial activity of herbal mixture extract against various bacteria.

Test pathogens	Diameter of zone of inhibition (mm) Hydro alcoholic mixture extract			
Solvents	**DMSO**		**Methanol**	
Gram positive	**50mg/ml**	**100mg/ml**	**50mg/ml**	**100mg/ml**
Bacillus subtilis	_	_	_	_
Bacillus cereus	8	15	12	14
Staphylococcus aureus	5	10	5	7
Staphylococcus epidermidis	16	18	5	6
Gram negative				
E.coli	6	12	5	12
Salmonella enterica	13	14	11	14

3.6. Polyherbal antidiabetic compressed tablet lozenges formulation and characterization

A number of herbal extracts are frequently used for the treatment of diabetes mellitus. *Tinospora cordifolia, Gymnema Sylvestre, Pterocarpus marsupium* and *Acacia arabica* extracts has been used medicinally since ancient times for diabetes. In the present study, lozenges containing hydro alcoholic extracts of these plants were made as appropriate for the patient to deliver the drug for diabetes therapy. At the time of dissolution of a solid drug form in the saliva, a solution of active substance is delivered in the mouth. The physicochemical characteristics of the drug form and the physiology of the oral cavity both influence the amount of drug in the mouth [29].

In the present work, compressed tablet lozenges were prepared using the wet granulation method. Other excipients that were used in the formulation of lozenges were sorbitol, mannitol, citric acid etc. PVP (polyvinyl-pyrrolidone) was used as a binder to hold together the discrete granules. Tablet hardness finally depends upon binder concentration, since the type and concentration of binder present will enhance the intragranular forces in each individual granule as well as intergranular forces, which are the bonding forces between granules. The tablet will break, leaving a rough and an irregular surface on the fracture line when intragranular force is greater than intergranular but if the intergranular forces are greater, the fracture will be smooth. Binders are effective in increasing the intergranular forces while at the same time helping to improve the surface texture characteristics of the lozenge when it is dissolving in the oral cavity [57]. Lubricants like magnesium

stearate, silica, PEG 6000 were also used which improves flow of final lozenges mixture or granules. The incidence of binding and picking of tablet during compression increases due to the addition of poly ethylene glycols (PEG) of low melting point (less than 50%) and low molecular weight in the formulation of lozenges. It also improves the organoleptic quality of the product. During the formulation of compressed tablet lozenges, the most important thing is the selection of flavours. Lemon flavour and menthol were also used to provide good taste and cooling effect. Sorbitol is better able to carry high quantities of active ingredients than the most excipients especially in a wet granulated tablet base, since formulation containing greater than 20% sorbitol tend to be tacky and adhesive with good compression characteristics but its hygroscopic nature makes it undesirable where extended shelf life is required or when moisture sensitive medicaments are incorporated in the granulation. Moisture resistant packaging is essential with sorbitol containing compressed lozenges. Mannitol is available as a fine powder for use in wet granulations. Mannitol contains less than 0.3% moisture and is nonhygroscopic. Its flow and compression characteristics are good, as are chemical inertness and resistance to discoloration [58].

3.6.1. Physicochemical characterization of herbal antidiabetic compressed tablet flozenges.

Lozenges tablets were evaluated for hardness, friability, thickness and disintegration time and found to be within the Pharmacopeia limits. The standard acceptable range for tablet hardness is 3-40 Kp. The hardness and thickness were found to be 20.3 kg/cm^2 and 5 mm respectively. The friability was found to be less than 1% i.e. 0.59%, which suggested a good potential for packaging and transportation. The moisture content was found to be 0.55% and disintegration time was 17 minutes.

Fig 3: Polyherbal antidiabetic compressed tablet lozenges.

During stability test, it was observed that there was no change in the physical appearance of the formulation (colour, taste, odour, texture). Tablets were evaluated for thickness, hardness, friability and disintegration time. As observed from the data, the formulation showed no significant changes in the hardness, thickness, friability and disintegration time when stored at 30°C and 40°C for a period of one month.

3.7. Sensory properties of antidiabetic herbal compressed tablet lozenges

The results presented in Fig. 4 showed the sensorial attributes of polyherbal antidiabetic lozenges. The studies on the colour, taste, odour and texture revealed that the polyherbal antidiabetic lozenges were the acceptable product.

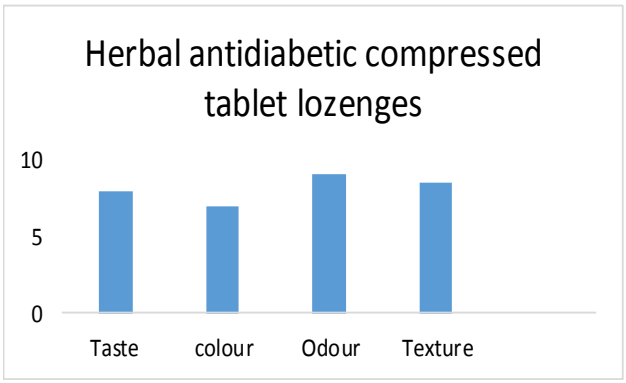

Fig 4: Sensory attributes of herbal antidiabetic compressed tablet lozenges.

4. Conclusion

There is increasing demand of natural products with antidiabetic activity. In the present study, medicated compressed tablet lozenges were made from extracts of plants having proven antidiabetic potential. Hydro alcoholic herbal extracts of *Tinospora cordifolia, Gymnema Sylvestre, Pterocarpus marsupium* and *Acacia arabica* were screened for phytochemicals and antioxidant potential. A significant amount of phenolics, flavonoids, saponins and tannins were present in extracts. Antioxidant activity by DPPH and FRAP assay was found to be higher in all the herbal extracts as well as in the mixture of herbal extracts. The existence of phytosterols and terpenoids, the largest group of phytochemicals, was indicated by qualitative tests. The herbal mixture extract possesses antibacterial activity with a significant zone of inhibition against *Bacillus cereus, Staphylococcus aureus Staphylococcus epidermidis, E. coli* and *Salmonella enterica.* HPLC analysis was carried out to identify bioactive compounds, with antidiabetic potential. Wet granulation method was used for preparation of lozenges and were evaluated for hardness, friability, thickness and disintegration time. Stability test indicated no significant changes in the physico-chemical parameters. Furthermore, was concluded that herbal antidiabetic lozenges were the acceptable product based on the sensory attributes. Here the present work creates a thrust for the future researchers for the conduct of clinical trials to confirm the efficacy of these plant extracts in the form of a lozenge for treatment of patient with diabetes.

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