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Research Article

In Vitro Studies on Antimicrobial Activity and Phytochemical Analysis of *Tinospora cordifolia* and use as Chocolate Supplement

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ABSTRACT

Background and Objectives: Day by day the antibiotic resistance is increasing and become a serious problem. Hence it's a today's need to find some naturally occurring bioactive compounds such as plant bioactive compounds. The objective of the study was to evaluate the antimicrobial activity of *Tinospora cordifolia* root extracts against human pathogens and *T. cordifolia* extract were tested for phytochemical examination. A chocolate product development with *T. cordifolia* extract was also developed. **Materials and Methods:** Antimicrobial efficiency of *Tinospora cordifolia*, a medicinal plants were examined using isopropyl alcohol, water, methanol, and acetone, as solvents and tested against four human pathogens like *Escherichia coli, Salmonella sp., Staphylococcus aureus, Pseudomonas auruginosa* using agar well diffusion method and minimum inhibitory concentration. The phytochemical analysis carried out for qualitative testing methods and *T. cordifolia* water extract was used as a supplement in chocolate preparation. **Results:** Water extract showed more extraction yield of 2.68%, followed by methanol (1.6%), isopropyl alcohol (1.13%) and acetone (0.58%). All the extracts showed significant activity against all pathogens, but the isopropyl alcoholic extract of *T. cordifolia* showed maximum zone of inhibition against all the microorganisms. Also, *S. aureaus* was inhibited by all extracts. Acetone extract was less potent to inhibit pathogens except *S. aureus*. The minimum inhibitory concentration of isopropyl alcohol, water, methanol and acetone extracts showed 3.48 mg, 8.04 mg, 3.9 mg and 1.74 mg, respectively. The phytochemical analysis carried out revealed the presence of alkaloids, carbohydrates, terpenoids, proteins, flavonoids, steroids, glycosides in most of the extracts of *T. cordifolia*. A chocolate preparation containing water extract of *T. cordifolia* was developed and can be available as source of medication for kids. **Conclusion:** The Spectrum of activity observed in the present study may be indicative of the present study extracts of *T. cordifolia* plants could be a possible source to obtain new and effective herbal medicines to treat infections, hence justified the ethnic uses of *T. cordifolia* against various infectious diseases.

Keywords: *Tinospora cordifolia; Giloy; Antimicrobial; Phytochemicals; Chocolate supplement*

INTRODUCTION

Antibiotic and antimicrobial agent resistance has become an increasingly important and booming global problem. These resistances were occurred due to the structural modifications of

antimicrobial drugs against various antimicrobial agents. These trends are going to create public health crisis and to avoid these crisis, there is need of substantial investment and research in the field of anti-infective agents. World Health Organization (WHO) has recommended development and use of environment friendly alternative methods to control various diseases[1]. Various medicinal plants have been clinically used in curing various human and animal disorders.

Natural products have been a particularly rich source of anti-infective agents i.e.; Giloy Satva (*Tinospora cordifolia)* known to have antimicrobial potential traditionally. *T. cordifolia* is an endangered and most versatile rejuvenative herb, which is popularly known as 'Guduchi'. *T. cordifolia* is an herbaceous shrub, well known for its medicinal properties. In Hindi *T. cordifolia* is called as Giloy. *T. cordifolia* is one of the most commercially exploited plants in biopharmaceuticals with an estimated annual demand of 10,000 Tones as a crude herbal drug in Indian System of Medicine[2].

Since the distant past, the whole plant showed diverse health benefits and has been used as traditional medicine against various human ailments. It is also called as 'Amrita or nectar of life', as acts as an immune stimulant in the body and maintains the functions of its various organs in harmony[3]. In the ancient literature *T. cordifolia* preparations were used in diabetes, fever, general weakness, urinary problems, dyspepsia, anaemia, skin diseases, viral hepatitis jaundice, chronic diarrhoea and dysentery. It has also been indicated to be useful in the treatment of leprosy, helmenthiasis, heart diseases and rheumatoid arthritis. More recently the *T. cordifolia* showed antimicrobial, antineoplastic, antioxidant, hypoglycemic, antipyretic, hepatoprotective, immunomodulatory, diuretic, antidiabetic, anti-stress, anti ulcer, digestive, mental disorder, anti-tuberculotic and antihyperglycemic properties have been reported by George et al.[4] and Dwivedi and Enespa[5]. All these beneficial medicinal effects of plant products typically result from the combination of secondary metabolites present in the plants and most of them were phenolics, flavonoids, alkaloids, and tannins[6]. The extract of stem of *T. cordifolia* revealed the presence of a wide range of phytoconstituents including alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, steroids and triterpenoids[7]. These phytochemicals are constituting antimicrobial activities and several reports showed *T. cordifolia* as a potent therapeutic agent[8-10].

Escherichia coli and other urinary tract infections (UTi) microorganisms caused diarrhea, dysentery and fever in baby infants and kids, immediately. Day by day incidences of infectious diseases are increasing and need to develop alternative medicines instead of new antibiotics. Importantly, most of the patients have medico phobia and are avoiding medication. It has been observed that the medication problems are behavioral and hence the present study was undertaken to examine the *in vitro* activity of *T. cordifolia* against pathogenic strain and its use as supplement in chocolate preparation.

MATERIALS AND METHODS

Materials and Chemicals

Tinospora cordifolia (local name: Giloe) stem powder was purchased from Nashik local market. The chemicals used for extractions were procured from Fisher Scientific (Mumbai, India).

Bacterial strains and Culture media

Bacterial cultures used for this study are *E. coli, S. aureus*, *Salmonella* sp., and *P. aeruginosa*. These cultures were maintained on nutrient agar slants at 4^0C and preserved as glycerol stocks at -20^0C. The cultivation and maintenance media were procured from Himedia Laboratories (Mumbai).

Preparation of Extract

T. cordifolia powder (10gm) was taken in 100mL different solvents (isopropyl alcohol, water, methanol and acetone) separately and kept for overnight period at room temperature. Next day the mixture was filtered through a cotton filter. It was dried on water bath until the constant weight with dried mass was obtained. All extracts were

stored in sterile glass bottles at room temperature until screened.

Antibacterial Activity

Screening of bacterial activity was carried out by agar well method on *E. coli*, *S. aureus*, *Salmonella* sp., and *P. aeruginosa*. Dried compound (10 mg mL^{-1}) was dissolved in DMSO solvent, and used 500µg as effective concentration to test its efficacy as antibacterial agent. Antibacterial testing was carried out by Agar well method on nutrient agar medium as described by Zambare and Bhoyte[11]. All plates (in duplicate) were incubated at 37°C for 48 h. The zone of inhibition by the extracted compound was measure in mm against DMSO as control[12].

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of all solvents extracts was measured by addition of increased concentration of extract followed by a loopful culture (1.0×10^7 CFU ml^{-1}) of test pathogen inoculation in nutrient broth. These tubes were incubated at 30^0C for 24 hrs and observed for turbidity. Tube concentrations having no turbidity were considered as MIC value of the extract for that pathogenic microorganism.

Qualitative Analysis of Phytochemicals

The extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in various extracts of *T. cordifolia*. The above phytoconstituents were tested as per the standard method[13,14].

Test for Alkaloids: To 1 ml of each extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed, appearance of dark orange or purple colour indicates the presence of alkaloids.

Test for Carbohydrates: Take 1 ml of extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the sample extract.

Test for Terpenoids: Take 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

Test for Proteins: To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.

Test for Tannins: To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

Test for Flavonoids: 2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for Saponins: To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

Test for Steroids: 2ml of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml Sulfuric acid. The colour changed from violet to blue or green indicate the presence of steroids.

Test for Glycosides: 2.5ml of the extract was mixed with a little quantity of anthrone on a watch glass, one drop of concentrated sulphuric acid was added and made into a paste, and heated gently over a water bath; a dark green colouration indicated the presence of glycoside.

Test for Phlobatannins: Deposition of a red precipitate when 2ml of extract of each plant samples was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Chocolate formulation

In a glass bowl weighed 100g of dark chocolate powder without any added favorer sweetening agent was taken and allowed to melt, by keeping bowl on steam bath to obtained 60 ml of chocolate syrup. Simultaneously 4% *T. cordifolia* stem powder boiled for 20 minute and filtered out through masculine cloth. Final 1% *T. cordifolia* concentration was mixed with chocolate syrup and then poured the mixer in chocolate mould followed by refrigerating under -20^{0}C for 10-15 min. The chocolates were removed from mould and packed in wrappers.

Data analysis

All data used for this experimentation is obtained from duplicate experiments. Standard deviation was calculated by Microsoft excel.

RESULTS

T. cordifolia is a plant material with numerous biological activities. Fig. 1, showing highest % yield obtained with water (2.68%) followed by methanol (1.30%), Isopropyl alcohol (1.16%) and acetone (0.58%).

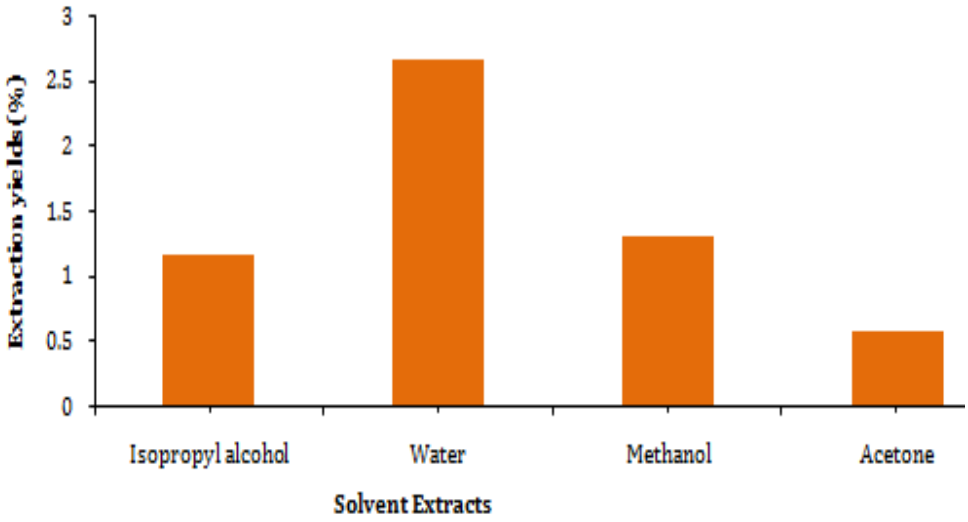

Fig. 1: Extraction yields of *T. cordifolia* in various solvent

Antibacterial Activity and MIC

Table 2 it was observed that, isopropyl alcohol inhibited all pathogens with maximum inhibition of *P. aeruginosa* (15.2±1.46 mm). Water extract inhibited *E. coli*, *S. aureus*, *Salmonella* sp and *P. aeruginosa*. Methanol extract inhibited *E. coli*, *S. aureus*, and *P. aeruginosa*. Acetone is the only extract which showed inhibition of only one pathogen *S. aureus* (14 ±1.22mm). Most of the extracts inhibited the pathogenic microbial cultures in range of 12-16 mm zone of inhibition.

Table 2: Screening of various solvent extracts of *T. cordifolia* for antimicrobial activities

Bacterial Name	Solvent Extracts and zone of inhibition (mm) ± SD			
	Isopropyl alcohol	Water	Methanol	Acetone
E. coli	12.6±0.89	0	15±0.63	0
S. aureus	12.6±0.89	12.2±0.83	12.6±0.48	14 ±1.22
***Salmonella* sp.**	12.6	14.2±1.09	0	0
P. aeruginosa	15.2±1.46	11.4±0.54	16.2±0.44	0

Table 3: Minimum inhibitory concentration (MIC) of various solvent extracts of *T. cordifolia*

Sr. No.	Extract Name	Pathogens and Minimum Inhibitory Concentration (MIC) Values (mg/ml)			
		E. coli	***S. aureus***	***Salmonella* sp.**	***P. aeruginosa***
1	Isopropyl alcohol	3.48	3.48	3.48	3.48
2	Water	0	8.04	8.04	8.04
3	Methanol	3.9	3.9	0	3.9
4	Acetone	0	1.74	0	0

S. aureus showed sensitive to all extracts followed by *P. aeruginosa*, *E. coli* and *Salmonella* sp. Table 3 showed Isopropyl alcohol as most potent extract which inhibits all test pathogens with a MIC value of 3.48 mg/ml. Water and methanol extracts showed MIC of 8.04 mg/ml (*S. aureus, Salmonella* sp., *P. aeruginosa*) and 3.9 mg/ml ((*E. coli, S. aureus, P. aeruginosa*), respectively. Acetone extract showing low profile antimicrobial activity but MIC value of 1.74 mg/ml with *S. aureus* showed a strong effect.

Phytochemcial Aanalysis

The phytochemical screening of *T. cordifolia* extracts shown presence of alkaloids, flavonoids, glycosides, steroids, terpenes, saponin, tannin, carbohydtrates and proteins which are important constituents of pharmacologically active phytochemicals (Table 4).

Table 4: Qualitative estimation of phytochemical in various solvent extracts of *T. cordifolia*

Sr. No.	Phytochemical	Solvent Extracts and Phytochemical Activities			
		IPA	**Water**	**Methanol**	**Acetone**
1	Alkaloids	+	+	+	-
2	Carbohydrates	+	+	+	-
3	Terpenoids	-	-	+	-
4	Proteins	+	+	+	-
5	Tannins	+	-	-	-
6	Flavnoids	-	+	+	-
7	Saponin	-	+	-	-
8	Steroids	-	-	+	-
9	Glycosides	+	+	+	-
10	Phlobatannin	+	-	-	-

+ Present, -Absent, IPA- Isopropyl alcohol

Chocolate supplementation

A dark chocolate with added *T. cordifolia* water extract showed all chocolate parameters from visibility, taste and flavor view points (Fig. 2).

Fig. 2: *T. cordifolia* water extract supplemented chocolate

DISCUSSION

Findings of the research clearly suggested that the *T. cordifolia* extract as a potential source of antimicrobial agent against human pathogens. Mishra et al.[12] showed sequential extraction of *T. cordifolia* stem with petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethyl alcohol, and water in Soxhlet apparatus. These results suggested that isopropyl alcohol extract is not only the important source of antimicrobial component but can also be used for developing novel antimicrobial biorationals of plant origin. Singh et al.[15] used five different solvents (ethanol, methanol, chloroform, dichloromethane, ethyl acetate) for antimicrobial activities and methanol crude extract of *T. cordifolia* showed best activity against *E. coli, S. aureus, P. aeruginosa, S. typhimurium* and *Candida albicans*. Singh et al.[16] investigated the plant (*T. cordifolia*) extracts viz., aqueous (TA), methanol (TM), and ethanol (TE) against uropathogens *E. coli* and *S. aureus*. This investigation showed relatively wide spectrum of antibacterial activity via zone of inhibition (ZOI) of ethanol extract (1.40 ±0.058cm to 1.77 ±0.30cm) against the test bacterial strains compared to methanol extract (1.20 ±0.100cm to 1.23 ±0.067cm). Salkar et al.[17] extracted leaves, stem and root of *T. cordifolia* in different solvents and tested against *E. coli, S. aureus* and *P. aeruginosa*. Here aqueous extracts were found to be effective against *E. coli* and *S. aureus* but *P. aeruginosa* was found to be very resistant to all these extracts. Bansal et al.[8] reported that the antimicrobial effect of different extracts of *T. cordifolia* against four pathogenic bacteria (*Escherichia coli, S. aureus, Streptococcus mutans* and *P. aeruginosa*) and one fungus (*Candida albicans*). Butanol extract was the most effective against all the tested microbes as compared to the other tested extracts.

Effective bactericidal activity of any compound was mainly judged by its minimum inhibitory concentration and it has to be as minimal as. Similar to our research findings but with different pathogens such as *Klebsiella pneumoniae* and *Pseudomonas* spp., Mishra et al.[12] found minimum inhibitory concentration (MIC) values of 1.29 and 22.73 mg/ml for acetone and ethyl acetate extracts of *T. cordifolia*, respectively.

Extract contains some bioactive compounds called phytochemicals. Various phytochemcials such as alkaloids, lactones, glyceroids, steroids, diterpenoids, phenolics, sesquiterpenoids, aliphatic compounds and polysaccharides were isolated and reported from *T. cordifolia* constituents[18]. However, Virmani et al.[10] reported presence of alkaloids, flavenoids, tannins, phenols, saponins, glycosides, amino acids and steroids in *T. cordifolia* extracts which were accountable for antimicrobial potential. A review article elaborated in detailed about morphological, phytochemical and pharmacology aspects of *Tinospora cordifolia* (Guduchi)[4]. All the parts of this plant reported for various ethnobotanical and therapeutic uses. It is prescribed for many diseases such as general debility, fever, diabetes, dyspepsia, urinary infections, jaundice and skin diseases**.** Gauniyal and Teotia[19], reported presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins reducing sugars, saponin phytochemcials in *T. cordifolia.*

The present study is the only initiative for making a chocolate with supplementation of phytophamaceutical extracts. Such supplemented product will not only change the patient's behavior but also shift the mind from medicophobic to medicophilic. Every kid, adults are very fussy for chocolate and a chocolate with medication is a excellent trends. Present study manufactured a chocolate with water extract of *T. cordifolia*. The *T. cordifolia* extract is bitter and kids are ignoring to take it. Supplementation of these extracts in *T. cordifolia* chocolate attracts the kids and the medication purpose will be solved. Dorle et al.[20] formulated an herbal gel with chloroform extract of *T. cordifolia* and was found excellent results of antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. Some formulation are already published by some authors such as a methanol extract of *T. cordifolia*

tablet[21] and a slow release medicament formulation containing *T. cordifolia, Gymnema sylvestre, Pterocarpus marsupium* and *Acacia Arabica* for diabetes treatment[22]. Thus, *T. cordifolia* extracts will have potential for development of various bio-formulations against various infectious diseases.

CONCLUSION

The study demonstrated the presence of various groups of phytochemicals in *T. cordifolia* extracts which are responsible for showing considerable antibacterial activities against urinary track infectious microorganisms. The chocolate formulation with *T. cordifolia* extract could be the best and easiest way of changing the behavioral way of medication phobia.

REFERENCES

1. WHO 2001. Antimicrobials in animal feed - threat to human use. WHO Drug Information, 15: 160-162.
2. Mamta, and Jakhar, K.K. 2016. Studies on *In vitro* antibacterial activity of *Tinospora cordifolia* stem extract on *Escherichia coli*. Vet. Res. Int., 4(2): 74-77.
3. Sarkar, B.K., Padhi, M.M., Vasanthakumar, K.G., Verma, S.C. and Deep, V.C. 2013. Evaluation of antimicrobial potential of traditional formulation Giloy Satva. Rasamruta, 5: 1-4.
4. George, M., Joseph, L. and Mathew, M. 2016. *Tinospora cordifolia*; a pharmacological update. The Pharma Innov. J., 5(7): 108-111.
5. Dwivedi, S.K. and Enespa, 2016. *Tinospora cordifolia* with reference to biological and microbial properties. Int. J. Curr. Microbiol. Appl. Sci., 5(6): 446-465.
6. Reddy, N.M. and Reddy, R.N. 2015. *Tinospora cordifolia* chemical constituents and medicinal properties: A review. Scholars Academic Journal of Pharmacy, 4(8): 364-369.
7. Mittal, J., Sharma, M.M. and Batra, A. 2014. *Tinospora cordifolia*: a multipurpose medicinal plant- A Review. J. Med. Plants Studies, 2(2): 32-47.
8. Bansal, D., Bhasin, P., Punia, A. and Sehrawat, A.R. 2012. Evaluation of antimicrobial activity and phytochemical screening of extracts of *Tinospora cordifolia* against somepathogenic microbes. J. Pharm. Res., 5(1): 127-129.
9. Khan, J.A. and Srivastava, R. 2012. A study on antibacterial properties of *Tinospora cordifolia* stem, root extracts. Int. J. Pharm. Res. BioSci., 1(6): 291-298.
10. Vermani, A., Navneet, and Gautam, S.S. 2013. Screening of antibacterial activity of *Tinospora cordifolia* Miers extracts against dental pathogens. J. Pharm, Toxicol., 8: 28-34.
11. Zambare, V.P. and Bhoyte, S. 2009. Antimicrobial activity of tea (*Camellia sinensis*). Biomed. Pharmacol. J., 2(1): 173-175.
12. Mishra, A., Kumar, S. and Pandey, A.K. 2013. Scientific Validation of the Medicinal Efficacy of *Tinospora cordifolia*. The Scient. World J., 1-8.
13. Trease and Evans (1989) Text Book of Pharmacognosy 12th ed., ELBS Publications. pp. 49, 126, 132-137, 205, 248.
14. Kokate, C.K., Khandelwal, K.R., Pawar, A.P. and Gokhale, S.B. 1995. Practical Pharmacognosy 3rd ed., Nirali Prakashan Pune: p.137.
15. Singh, G., Saxena, R.K. and Singh, N.K. 2016. Screening of potential antimicrobial activity of Indian medicinal plant of different solvent extract: *Tinospora cordifolia* and *Hymenocallis littoralis*. Int. Res. J. Eng. Technol., 3(3): 928-932.
16. Singh, P., Katiyar, D., Singh, B. and Srivastava, A. 2015. Antimicrobial activity of *Tinospora cordifolia* extracts against urinary tract infections causing bacteria. Int. J. Pharma BioSci., 6 (3): 571 – 577.
17. Salkar, K., Chotalia, C. and Salvi, R. 2017. *Tinospora cordifolia*: An Antimicrobial and Immunity Enhancer Plant. Int. J. Sci. Res., 6(3): 1603-1607.

18. Gopi, M., Shrikumar, S. and Ravi, T.K. 2004. *Tinospora cordifolia* (Amrita): an important Ayurvedic plant. Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbtore, http://www.pharmainfo.net/reviews/inospora-cordifolia-amritha-%E2%80%93-important-ayurvedidic-plant.
19. Gauniyal, P. and Teotia, U.V.S. 2014. Phytochemical screening and antimicrobial activity of some medicinal plants against oral Flora. Asian Pac. J. Health Sci., 1(3): 255-263.
20. Dorle, A.D., Swami, K.S., Nagare, S.K. and Hyam, S.R. 2015. Design and evaluation of novel topical gel of *Tinospora cordifolia* as antimicrobial agent. Asian J. Pharm. Clin. Res., 8(6): 237-239.
21. Mehra, R., Naved, T., Arora, M. and Madan, S. 2013. Standardization and evaluation of formulation parameters of *Tinospora cordifolia* tablet. J. Adv. Pharm. Edu. Res., 3(4): 440-449.
22. Yadav, S., Nand, P. and Gupta, R.K. 2015. Formulation and phytochemicals characterization of polyherbal (*Tinospora cordifolia*, *Gymnema sylvestre*, *Pterocarpus marsupium* and *Acacia arabica*) antidiabetic compressed tablet lozenges. J. Pharm. Phytochem., 4(2): 244-253.