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Asem Robinson Singh
EMRC, Hengbung, Manipur, India

Thangjam Davis Singh
EMRC, Hengbung, Manipur, India

Leivon Edwin Kom
EMRC, Hengbung, Manipur, India

LD Rovei
EMRC, Hengbung, Manipur, India

Dhanaraj Singh Thokchom
EMRC, Hengbung, Manipur, India

AKS Rawat
EMRC, Hengbung, Manipur, India

Correspondence
Dhanaraj Singh Thokchom
EMRC, Hengbung, Manipur, India

Evaluation of hypoglycemic and antioxidant activities of 'Koubru Yai' (*Stephania rotunda* Engl.): An important medicinal plant used in traditional medicine of Manipur

Asem Robinson Singh, Thangjam Davis Singh, Leivon Edwin Kom, LD Rovei, Dhanaraj Singh Thokchom and AKS Rawat

Abstract
The traditional practices documented during plant survey conducted in the state of Manipur region revealed the use of tuber of *Stephania rotunda* (SR) for treatment of diabetes by the local communities. The present study was aimed to evaluate hypoglycemic and antioxidant activity of ethanol and water extracts of SR tuber. Water and alcoholic extract of SR tuber were compared for total phenolic, flavonoids, antioxidant and α-amylase inhibition activity. The alcoholic extract of *Stephania rotunda* having high concentration of total Phenols and Flavonoid showed significant α-amylase inhibition activity (IC_{50} = 78.06µg/mL) and also antioxidant activities (DPPH) as compared to water extract. The present study provides the evidence that the use of *Stephania rotunda* tubers help in maintaining blood sugar level by the local communities of Manipur. Further, this study will also provide leads for future drug development programme for amelioration of diabetes.

Keywords: antioxidant, hypoglycemic, *Stephania rotunda* Engl, manipur, traditional medicine

Introduction
There is world-wide interest towards the use of herbal medicine for different ailments and well-being. The herbal medicine specially, traditional medicine, provide not only healthcare to a large population but also give new leads for development of phytomedicine through identification of new bioactive molecules and their derivatives. A large number of such molecules *viz*., artimisinin, metformin, vincristine, ephedrine, taxol, L-dopa, forskolin etc. were derived from plants which have been used by different communities as traditional-medicine or folklore medicaments. In India, a large ethnic communities uses local plant biodiversity for their healthcare and as food which has nutraceutical benefits as well as medicinal values to control various ailments such as diabetes.

Presently, diabetes is one of the most common metabolic disorder in the world as it is evident from the WHO Global Report on Diabetes 2016, that globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. In India, according to the International Diabetes Federation (IDF) there were over 72 million cases of diabetes in India in 2017 with rising rate 6.5% [1]. Therefore, there is an urgent need to combat this threat of diabetes by developing effective herbal formulations and identifying new novel phyto-molecules taking leads from traditional medicine which has been used effectively since generations.

The north eastern (NE) region of India falls in the one of the global hotspot of biodiversity. The NE states of India including the state of Manipur are rich in plant diversity and ethnic communities having vast traditional knowledge about the diverse uses of medicinal plants and thus there are considerable scope for exploitation for new phyto-medicine specially for metabolic and infectious diseases. Manipur is situated in the NE border of India and lies between 23°83´N and 25°68´N latitudes and 93°03´E to 94°78´E longitudes. The state covers a total geographical area of 22,347 sq.km of which about 91.75% is covered by the mountains and the Central Valley about 8.25%. The NE states have been surveyed extensively for documentation of its diverse plant wealth and traditional medicine. a survey carried out by Khumbongmayum *et al.* (2004) [2] in four sacred groves of Manipur revealed that traditionally 120 plant species representing 106 genera and 57 families having therapeutic applications.

These plants from the sacred groves have been used by the medicine-men (*Maiba*) for preparation of traditional medicine. Another survey conducted by Das and Tongbram (2014) revealed that the Meitei community in Bishnupur district of Manipur traditionally uses about 106 plant species belonging to 55 families in curing different ailments. It was reported that the Meitei community still believes in the traditional use of herbal medicine and therefore, conserve certain medicinal plants in their home kitchen gardens [3].

During our plant survey (2016-17) of six districts of Manipur, 72 most important plant species were documented for the treatment of various ailments viz asthma, burn, cold and sore throat, chest and arm pain, cough, diarrhea, gastric, headache, fever, skin infection, piles and high sugar etc. Out of these plants reported by local communities, tuber of *Stephania rotunda* is used frequently for the treatment of high blood sugar. One of the plants used is the. Various medicinal properties such as anticancer, anti-inflammatory, febrifuge, antidiabetic etc. were reported by Chopra *et al.* (1958) [4]. In Ayurvedic system of medicine the tuber of *S. rotunda* is also reported to have antidiarrheal, analgesic, antihelminthic, febrifuge, ability to correct cardiac disorders, skin disorders etc [5].

The plant of *Stephania rotunda* (SR) belongs to Family Menispermaceae a native to eastern and southern Asia and Australasia. Plants are herbaceous, perennial with vines growing to around four metres tall, with a large, woody caudex. The leaves are arranged spirally on the stem, and are peltate, with the leaf petiole attached near the centre of the leaf.

Comprehensive information on chemical & pharmacological activities on genus *Stephania* including *S. rotunda* was reviewed by Semwal *et al.* (2010) [6]. Over 200 alkaloids together with flavonoids, lignans, steroids, terpenoids and coumarine have been identified in the genus *Stephania*. Later Desgrousas *et al.* (2014) [7] recorded traditional uses of different parts of *S. rotunda* in Southeast Asia countries (Cambodia, Vietnam, Laos, and India) to treat a wide range of ailments, including asthma, headache, fever and diarrhea and identified forty alkaloids. The roots primarily contain L-tetrahydropalmatine, whereas tubers contain cepharanthine and xylopinine. The alkaloids exhibited different major pharmacological activities *viz.*, anti-plasmodial, anti-cancer and immunomodulatory effects [7]. Apart from established phytocompounds used in modern allopathic medicines there are a number of extracts that has potentials of ameliorating various ailments. Examples of some of the plants of traditional medicine and scientifically validated antidiabetic properties are *Ipomoea* sp [8] *Aegle marmelos* [9], *Allium hookeri* [10] etc. Similarly, *Stephania rotunda*, though used as a traditional medicine by native people of Manipur, hasn't been scientifically validated for its antidiabetic properties although studies has been carried out in other genus members of the species.

Present study was undertaken with the same objective to evaluate alcohol and water extracts of *Stephania rotunda* tuber for hypoglycemic activity along with antioxidant activity which is being used by local Manipur communities. Further, to develop HPTLC fingerprint of potential extract for quality assurance.

Materials and Methods

Plant collection- The tubers of *S. rotunda* were collected from the forest of Hengbung, Kangpokopi, Manipur and after taxonomically identification herbarium specimens were deposited in Ethnomedicinal Research Centre with No EMRC-0021.

Extract preparation and determination of extractive values

The plant tubers were washed in running water and cut into small bits to facilitate drying. The pieces of tuber were shade dried. The dried plant materials was taken separately and ground using an electric blender and sieved at 60 mesh size to obtain a fine powder. The powdered samples were stored in a clean glassware container until needed for analysis.

Maceration of the plant sample was carried out in solvents viz. Ethanol and Water. Hundred grams of dried powder was taken and soaked in 500 ml of respective solvents for 18 hours in a 1000 ml conical flask. The extracts were then concentrated using rotary vacuum evaporator to get ethanol extract (SRA) and water extract (SRW) and later its weights were determined after complete removal of solvents.

Estimation of total phenolics

Amount of total phenolics in the plant extracts were determined using Folin-Ciocalteu phenol (FC) reagent by the method of Singleton and Rossi (1965) [11] with slight modification. Equal quantities of 0.4 mL of 60% ethanol and aqueous extract of rhizomes (10× dilution) were mixed with 0.3 mL of FC reagent (10×dilution). Then, 0.25 mL of sodium carbonate (7.5% w/v) was added after 3 min and total volume was made up to 4 mL with distilled water. The reaction solution was kept in dark for 30 min. Absorbance was measured at 765 nm against a blank. The flavonoid experiment was carried out in triplicates and the amount of total phenolic content was calculated as gallic acid equivalent (GAE) in mg/100g. The concentration range of gallic acid used for standard curve was 2-10 µg/mL.

Total flavonoids estimation

Total flavonoid content of the plant extracts were determined by the colorimetric method as described by Jia *et al.* (1999) [12] with minor modification. Equal quantities of 0.5 mL of rhizome extracts prepared in 60% ethanol and aqueous were mixed with 2 mL of 30% ethanol and 0.15 mL of 5% $NaNO_2$. After 6 min, 0.15 mL of 10% Al $(NO_3)_3$ was added. Subsequently, 2 mL of 4% NaOH and 0.2 mL of 30% ethanol were added to the reaction mixture after 6 min and mixed thoroughly. Absorbance was recorded at 510 nm following an incubation of 12 min against a blank. All experiments were carried out in triplicates and expressed as Quercetin equivalent (QE) in mg/100g of the extracts.

HPTLC fingerprinting of extracts

Preparation of extract for HPTLC: The dried sieved powder of SR tuber was extracted for 8hrs with ethanol and water separately later filtered with Whatman No.1 filter paper. The filtrates thus obtained was concentrated in a rotary evaporator at 40 °C and lyophilized. The dried extracts were dissolved in ethanol and water respectively to obtain stock solution of 10mg/ml, which is used for application of spots on HPTLC plate.

Instrumentation and Chromatographic Conditions: The following were the instruments and chromatographic conditions used. Spotting device: Linomat V automatic sample applicator; CAMAG (Muttenz, Switzerland), Syringe: 100 µL Hamilton (Bonaduz, Switzerland). HPTLC chamber: glass twin trough chamber (20 × 10 × 4cm); CAMAG. Densitometer: HPTLC Scanner 3 linked to win CATS

software V.4.06; CAMAG.HPTLC. plates: 20 × 10 cm, 0.2 mm thickness precoated with silica gel 60 F254; E. Merck (Darmstadt, Germany). Experimental conditions: temperature, 25±2°C; relative humidity, 40%. Solvent system: N-propanol; water: formic acid; 9.8:0.2:0.04 v/v/v. Detection of bands was carried out at wavelength of 366 nm.

Evaluation of antioxidant activity (DPPH Assay)-

DPPH assay was carried out to assess the free radical scavenging capacity of the plant extracts following the method of Miliauskas *et al.* (2004) [13] with slight modification. Each reaction mixture consisted of 100 µM DPPH (2, 2-diphenyl-1-picrylhydrazyl) in absolute methanol with different concentrations of sample. Absorbance was read against a blank at 515 nm after incubation of the reaction mixtures for 30 minutes in dark at room temperature. The percentage of DPPH decolouration was calculated as follows:

Percent DPPH decolouration = [Abs control - Abs sample / Abs control] × 100.

The degree of discoloration of purple colored DPPH to yellow color signifies the free radical scavenging capacity of the extract. Ascorbic acid was used as standard compound. The IC_{50} value was determined from the DPPH discoloration curve.

***In vitro* antidiabetic activity (alpha amylase inhibition assay) -**

Assay was carried out with slight modification based on the starch-iodine test described by Xiao *et al.*., 2006 [14]. Ethanol and water extract (500 µL) of varied concentrations were added to 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37°C for 10 min, then 500 µL soluble starch (1% w/v) was added to each reaction well and again incubated at 37°C for 15 min. 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µL of iodine reagent (5 mM I_2 and 5 mM kI). The colour change was noted and the absorbance was read at 620 nm on a micro plate reader. The control reaction representing 100% enzyme activity did not contain any plant extract to eliminate the absorbance produced by plant extract; appropriate extract controls without the enzyme were also included. Inhibition of enzyme activity was calculated as: Inhibition of enzyme activity (%) = (C-S) / C × 100.

Where, S is the absorbance of the sample and C is the absorbance of blank (no extract).

Statistical analyses

All statistical analyses were conducted using Graph Pad Prism 6.0. Independent sample t-tests were used to compare between SRA and SRW extract values and linear regression analysis was conducted to determine significance of dose dependence of enzyme inhibition within doses of SRA or SRW. Pearsons correlation was employed to estimate correlation between phenolics or flavonoid content with DPPH discoloration. P value of less than 0.05 (95% CI) was considered statistically significant.

Results

Extractive values

Dried powder (100g) of SR tuber was used for extraction in alcohol and water. The weights of the extracts obtained was more in SRA with an average yield of 1.37± 0.26g compared to SRW which yielded 1.20±0.07g. An independent t-test was conducted if the yield in SRA is significantly higher from SRW which revealed no statistical significance (p = 0.132). The result suggests that SRW and SRA yielded almost equivalent amount of extracts.

HPTLC and phytochemical analysis

The bands observed in SRA extract was different from those observed in SRW extract. The presence of a prominent band with Rf value of 0.11, belonging to berberine group of compounds, was observed in both the extracts. Additionally, some of the major bands observed in both extracts which has light green fluorescence with Rf value of 0.23, a light blue band observed in SRA extract with a Rf value of 0.37 which is absent in SRW extract, a bluish green band at Rf value of 0.57 which was significantly rich in SRA extract but poorly visible in SRW extracted sample, another distinct band of blue color was observed with Rf value of 0.67 in the SRA extract but faintly visible in SRW [Figure 1, Table 1].

Table 1: Rf values of major compounds detected in SRA and SRW

R_f value	Color of the band (UV 366nm)
0.11	Yellow
0.23	Light green
0.37	Light blue
0.57	Bluish green
0.67	Blue

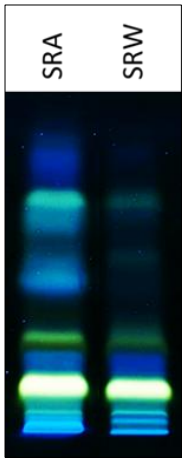

Fig 1: HPTLC profile of Alcohol and Water extract of S. rotunda. Under UV 366nm, solvent system N propanol: water: formic acid; 9.8:0.2:0.04 showing the presence of Berberine group.

SRA being extracted by a less polar solvent seems to contain a larger number of compounds compared to the SRW fraction which suggests that the tuber of SR contains more non-polar molecules than polar ones.

Total phenolics and flavonoids content in *S. rotunda* (SR)

Qualitative estimation of flavonoids was performed using quercetin as standard and calculated as mg equivalent (QE) per 100g of dried sample. The flavonoid content of SRA seems to be significantly higher with 2611.5±40 mg QE/100g dried sample compared to 1648.5±41 QE/100g dried sample weight. On the contrary, the phenolics content measured in gallic acid equivalent (GAE), is higher for SRW with 1145±41 mgGAE/100g compared to 953±25 mg GAE/100g dried sample [Figure 2].

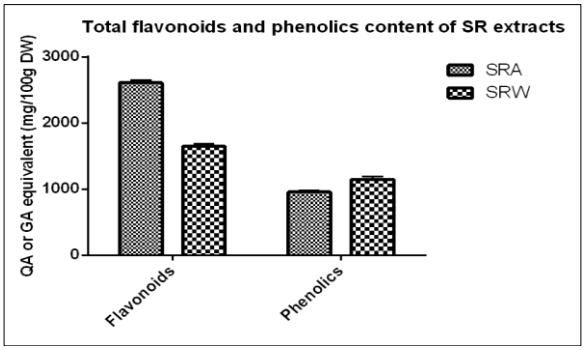

Fig 2: Total phenolic and flavonoids content of S. rotunda tuber extracted with Alcohol, and water. Alcohol extract showed the highest phenolic content. Gallic acid and Quercetin were used as standards.

Antioxidant property of *S. rotunda*

Estimation of antioxidant capacity of SRW and SRA by DPPH discoloration assay revealed that SRA has a much better antioxidant potential compared to SRW [Figure 3]. SRA showed an IC_{50} of 212.29 µg/ml whereas SRW showed an IC_{50} value of 257.66 µg/ml against an IC_{50} value of 3.2 µg/ml for ascorbic acid. The antioxidant capacity of SR tuber extracts were not found to be significantly strong than the standard ascorbic acid.

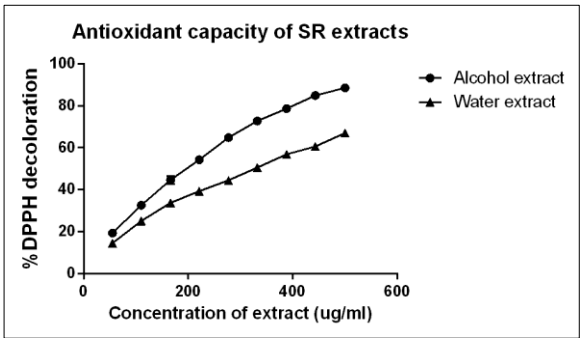

Fig 3: Antioxidant capacity of SRA and SRW estimated through DPPH discoloration assay. Ascorbic acid was used as a control for comparison.

Since SRA showed better antioxidant capacity than the SRW, we tried to establish a correlation between the phenolics or flavonoid content and the ability to discolor DPPH solution. A Pearson correlation was established between the three variables which yielded a statistically significant result. Content of flavonoids in SRA seems to better determine the antioxidant capacity ($p = 0.01$) than the content of phenolics ($p = 0.03$), though both are of statistical significance (Pearson $r = 0.99$) [Figure 4].

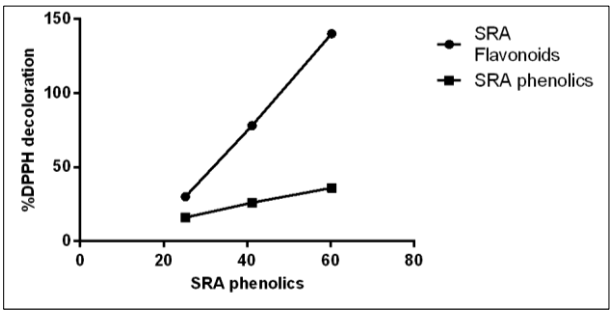

Fig 4: Correlation between flavonoid and phenolics content and percent DPPH discoloration of SRA.

Antidiabetic activity of SRW and SRA

A dose dependent increase in α-amylase inhibitory activity was observed in both SRA and SRW extracts. Linear regression analysis of dose dependent response of SRA and SRW on inhibition of α-amylase activity yielded high level of statistical significance ($p \leq 0.00$). The ethanol extract of the plant sample showed better bioactivity with an IC_{50} value of 78.06µg/mL compared to 97.48µg/mL seen in water extract [Figure 5]. Acarbose was used as a positive control with IC_{50} of 33.06 µg/mL.

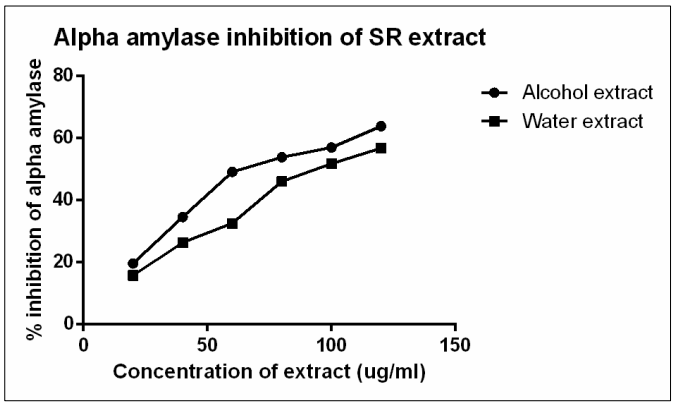

Fig 5: Alpha amylase inhibition potential of SRA and SRW extracts. SRA showed better inhibition potential compared to SRW.

Discussion

Traditional knowledge for use of plants for treatment of various ailments by different ethnic groups in the world are goldmine for search of novel bioactive compounds The present study reveals that tubers of *S. rotunda* which are being used by local communities of Manipur for the treatment of high blood sugar (Hyperglycemia) as alcoholic extract showed significant α-amylase inhibition activity. The IC_{50} of alcohol extracts was significantly higher as compared to that of water extract. The high hypoglycemic activity of alcohol extract may be due to high content of total Phenolics and Flavonoids content [Figure 2]. The α-amylase inhibition activity is also one of the parameter to assess the potential of hypoglycemic activity as reported by earlier workers in plant species [15]. Earlier workers also reported tuber of *S.gabra*, *S. tetrandra* and *S. hernandifolia* which are also used by different tribes of NE India [4, 6] were also reported to have hypoglycemic activity [16]. Semwal *et al.*., 2010 documented the antihyperclycemic activity of *S. gabra* to the palmatine derivatives obtained from the tuber of the plant. In China a number of ethnic communities are also uses the tubers of *Stephinia* species for various ailments [17]. Earlier workers have experimentally demonstrated antioxidant [18, 19] properties but hypoglycemic activity in *S. rodunda* is not reported. There are some common compounds within the different species of *Stephania* genus which might be responsible for the similar activity viz., hypoglycemic, and therefore, its similar use have been reported in different communities.

Estimation of extracted values determine the amount of phytochemicals that are present in a specific solvent which ultimately determine the quality of raw drugs. In case of *S. rotunda* tuber the alcohol extractive values were found to be highest 13.7±2.6 % followed by water extract with 12±0.7%. Further, quantitative estimation presence of phyto-chemical in different extracts revealed that total Phenolic content (1145±41 mgGAE/100g) is higher in SRW and Total Flavonoid content (2611.5±40 mgQE/100g) were higher in

ethanol extract using Gallic acid and quercetin as standard to estimate equivalence. Thus suggesting that ethanol extract having high content of Flavonoid may have higher biological activity.

HPTLC fingerprinting has been used successfully by several workers for quality assurance of number of medicinal plants such as *Coleus forskohlii* [20], *Adiantum species* [21], *Berberis* species [22]. HPTLC studies of ethanol and water extracts shows four common fluorescence bands at Rfs 0.23, 0.37,0.57 & 0.67 and a major band observed at Rf 0.11 showing yellow fluorescence under 365nm (an alkaloid of Berbirine group) [23] in these extracts. These HPTLC fingerprints of different extracts may be helpful for quality assessment of the *S. rotunda* tuber.

The correlation of antioxidant activity of alcoholic extracts with phenolics and flavonoids content revealed that flavonoid and phenolics both contributed towards the antioxidant property of the extract, but more prominently by the flavonoids of SRA. These further corroborates the earlier studies on antioxidant capabilities of flavonoids in other plant species [24, 25]. Further, the strong α-amylase inhibitory property of SRA suggests that the extract contains more bioactive molecules that can function as antidiabetic molecule through prevention of degradation of starch into glucose thereby limiting absorption by the intestinal wall. A number of FDA approved drugs such as acarbose [26] has similar function and SR extracts can be used as one of the potent phytochemicals for control of hyperglycemia.

Conclusion

The present study indicate and support the use of *S. rotunda* tubers for maintaining blood sugar level by local communities of Manipur as it has hypoglycemic activity through inhibition of α-amylase. The alcoholic extract, as compared to water extract has a better biological activity and also has high concentration of total Phenols and Flavonoids. The study provides leads for exploitation of *Stephania rotunda* tuber for search of new phyto-medicine for treatment of diabetes.

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References

1. International Diabetes Federation. Members 2018 [cited 2018 24 July]. Available from: https://www.idf.org/our-network/regions-members/south-east-asia/members/94-india.html.
2. Khumbongmayum AD, Khan M, Tripathi R. Sacred groves of Manipur–ideal centres for biodiversity conservation. Current Science. 2004; 87(4):430-3.
3. Das AK TY. Study on medicinal plants used by Meitei community of Bishnupur district, Manipur. International Journal of Current Research 2014; 6(2):5211-9.
4. Chopra R. Chopra's Indigenous Drugs of India, Calcutta: UN Dhur and sons Pvt. Ltd, 1958.
5. KC C. Bhavaprakash nighantu. Varanasi: Chaukhamba Bharati Academy. 1999:3.
6. Semwal DK, Badoni R, Semwal R, Kothiyal SK, Singh GJP, Rawat U. The genus Stephania (Menispermaceae): Chemical and pharmacological perspectives. Journal of ethnopharmacology. 2010; 132(2):369-83.
7. Desgrouas C, Taudon N, Bun S-S, Baghdikian B, Bory S, Parzy D, *et al.*. Ethnobotany, phytochemistry and pharmacology of Stephania rotunda Lour. Journal of ethnopharmacology. 2014; 154(3):537-63.
8. Pandey AK, Gupta PP, Lal VK. Preclinical evaluation of hypoglycemic activity of Ipomoea digitata tuber in streptozotocin-induced diabetic rats. Journal of basic and clinical physiology and pharmacology. 2013; 24(1):35-9.
9. Dhanaraj Singh T, Asem Robinson S. Ethnopharmacological Relevance of Aegle marmelos (L.) Correa for Treatment of Diabetes Mellitus: A Review. Int J Pharm Sci Rev Res. 2018; 48(2):15-9.
10. Singh KD, Chetia D, Mazumdar S. Anti-diabetic activity of methanolic extract of Allium hookeri leaves. Indo Am J Pharm Sci. 2013; 3:4098-104.
11. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965; 16(3):144-58.
12. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry. 1999; 64(4):555-9.
13. Miliauskas G, Venskutonis P, Van Beek T. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chemistry. 2004; 85(2):231-7.
14. Xiao Z, Storms R, Tsang A. A quantitative starch? Iodine method for measuring alpha-amylase and glucoamylase activities. Analytical biochemistry. 2006; 351(1):146-8.
15. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC complementary and alternative medicine. 2011; 11(1):5.
16. Semwal DK, Rawat U, Semwal R, Singh R, Singh GJP. Anti-hyperglycemic effect of 11-hydroxypalmatine, a palmatine derivative from Stephania glabra tubers. Journal of Asian natural products research. 2010; 12(2):99-105.
17. Xie D, He J, Huang J, Xie H, Wang Y, Kang Y, *et al*. Molecular phylogeny of Chinese Stephania (Menispermaceae) and reassessment of the subgeneric and sectional classifications. Australian systematic botany. 2015; 28(4):246-55.
18. Gülçin İ, Elias R, Gepdiremen A, Chea A, Topal F. Antioxidant activity of bisbenzylisoquinoline alkaloids from Stephania rotunda: cepharanthine and fangchinoline. Journal of enzyme inhibition and medicinal chemistry. 2010; 25(1):44-53.
19. Bory S, Bun SS, Baghdikian B, Dumètre A, Hutter S, Mabrouki F, *et al*. HPLC analysis of Stephania rotunda extracts and correlation with antiplasmodial activity. Phytotherapy research. 2013; 27(2):278-84.
20. Srivastava SK, Chaubey M, Khatoon S, Rawat A, Mehrotra S. Pharmacognostic evaluation of Coleus forskohlii. Pharmaceutical biology. 2002; 40(2):129-34.
21. Singh S, Khatoon S, Singh H, Behera S, Khare P, Rawat A. A report on pharmacognostical evaluation of four Adiantum species, Pteridophyta, for their authentication and quality control. Revista Brasileira de Farmacognosia. 2013; 23(2):207-16.

22. Andola HC, Rawal R, Rawat M, Bhatt I, Purohit VK. Analysis of berberine content using HPTLC fingerprinting of root and bark of three Himalayan Berberis species. Asian J Biotechnol. 2010; 2(4):239-45.
23. Rojsanga P, Gritsanapan W, Suntornsuk L. Determination of berberine content in the stem extracts of Coscinium fenestratum by TLC densitometry. Medical Principles and Practice. 2006; 15(5):373-8.
24. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radical Biology and Medicine. 2006; 41(12):1727-46.
25. Arts MJ, Haenen GR, Wilms LC, Beetstra SA, Heijnen CG, Voss H-P, *et al.* Interactions between flavonoids and proteins: effect on the total antioxidant capacity. Journal of agricultural and food chemistry. 2002; 50(5):1184-7.
26. Chiasson J-L, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M, *et al*. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. The Lancet. 2002; 359(9323):2072-7.