Phytochemical and antioxidant evaluation of Urginea indica Kunth.

Article *in* Indian journal of traditional knowledge · September 2018

CITATION
1

READS
382

3 authors, including:

Dipa Mahato
NISCAIR
4 PUBLICATIONS **5** CITATIONS
SEE PROFILE

Hanuman Prasad Sharma
Ranchi University
29 PUBLICATIONS **105** CITATIONS
SEE PROFILE

Indian Journal of Traditional Knowledge
Vol. 17 (4), October 2018, pp 783-788

Phytochemical and antioxidant evaluation of *Urginea indica* Kunth.

Dipa Mahato*, Abhishek Prasad Sahu & HP Sharma

Lab of Biotechnology and Plant Physiology, Department of Botany, Ranchi University, Ranchi-834 001, Jharkhand, India
E-mail: dipamahato@gmail.com

Received 27 February 2018, revised 18 July 2018

Urginea indica Kunth. is a rare, threatened and endangered medicinal plant that belongs to Liliaceae family and commonly known as *Jangli Pyaz*. In the present study, phytochemical analysis and antioxidant activity of the methanolic extract of *Urginea indica* bulbs was carried out. The secondary metabolites produced by this medicinal plant are reported to have therapeutic values. The secondary metabolites like phenol, flavonoid, tannin, saponin and alkaloid have been analyzed qualitatively as well as quantitatively in this species. The quantitative estimation has revealed the highest concentration of tannins (130.10 mg of GAE/g) in methanolic extract of *Urginea indica* bulb, whereas alkaloids (17.80 mg/g of dry plant sample), flavonoid (13.66 mg of QE/g), phenol (6.27 mg of GAE/g) and saponin (4.00 mg/g of dry plant sample) were found to be in good quantity. Considering the importance of natural products in modern phytomedicine, the antioxidant activity of *Urginea indica* extract was evaluated. The methanolic extract showed antioxidant activity by DPPH assay (IC_{50} = 51.87 µg/mL) comparable to gallic acid (IC_{50} = 39.91 µg/mL). Such an effect might contribute to explaining the traditional use of wild onion sps, *Urginea indica* in the treatment of various chronic diseases.

Keywords: Methanolic extract, Secondary metabolites, Antioxidant activity, Phytomedicine, Gallic acid

IPC Int. Cl.[8]: C09K 15/00, A61K 36/00, A61K 39/395, A61K 48/00, C07, C08, C23C, C11C, C10G

Plants have long been used by men for their basic needs. In essence, practices of medicinal plants are deeply rooted in the society of indigenous community, and the traditional knowledge passes from generation to generation, verbally. A plethora of evidence reflected the therapeutic significance of plants in India as well as in other countries[1-3]. In fact, medicinal plants are considered to be the main sources of several phytochemical compounds (PCs) like alkaloids, tannins, phenols, steroids and flavonoids curing diversified chronic diseases[4]. The most obvious role of PCs is protection from free radicals or reactive oxygen species (ROS) that is produced continuously in human body[5]. In this context, *Urgenia indica* Kunth. plays a major role, having multidisciplinary medicinal value.

Urginea indica Kunth. is a rare, threatened and endangered medicinal plant that belongs to Liliaceae family and commonly called as sea onion or *jangli pyaz* in India[6]. *Urginea* is one of the extremely interesting polytypic genera with about 100 species and is represented in India by nine species[7,8]. Some species of this genus have toxicological effects. They are also used as raticide and insect repellent[9]. To detoxify this toxic effect of the plant, people in different regions used several ways to detoxification of this herb before medicinal application. In the state of Jharkhand this species is wildly found in forest regions. The bulb of this plant is eaten by tribal people after detoxifying the bulb by soaking in rain water then boiling with water[10,11]. Its bulbs are excellent source of medicine with pharmaceutical and therapeutic applications mainly as anticancer, expectorant, cardiac stimulant, used in hypertension, dyspepsia, arterio-sclerosis, in treatment of asthma, rheumatism, edema, dropsy, allergies, gout, wound healing and to treat various other ailments[12]. In a recent study *U. indica* was reported to show antiangiogenic and proapoptotic activity[13]. Therefore, the objective of the present study is to determine the qualitative and quantitative estimation of total phenolic, flavonoid, tannins, alkaloids and saponins content in the bulb of the *U. indica* plant as well as to find out its antioxidant activity.

Material and methods

Plant material

The plant material of *Urginea indica* was collected from the well botanically leveled medicinal garden of Birsa Agriculture University (BAU), Ranchi in 2017. The Botanical identity of the plant was confirmed by

*Corresponding author

Professor HP Sharma, comparing with the authentic samples at the Herbarium of Taxonomy Department of Ranchi University, Ranchi. The voucher specimen No. of the plant is HPS/DM/151. The bulbs were separated from the plant and washed carefully. The washed bulbs were cut into small pieces and shade dried. After 3 weeks, when bulbs pieces completely dried, they were ground into powder form.

Extraction of plant material

The shade dried plant powder was soaked with methanol (1:10 ratio). The flask was covered with aluminum foil to avoid evaporation and then kept for 48 h in shaker incubator. After 48 h the solution was filtered by using Whattman filter paper No.1 and the filtrate was collected in a beaker. Then the filtrate was kept in incubator at 37 °C to evaporate the solvent. The prepared extract was then stored at 4 °C for further use[14].

Phytochemical screening

Phytochemical screening was carried out on the aqueous extract of powdered sample using standard procedures to identify the constituents, *viz*. Phenol, Flavonoid, Tannin, Saponin and Alkaloid[15]. A preliminary phytochemical screening was carried out as per the standard methods described by Harborne and Evans[16,17].

Phytochemical estimation

Determination of total phenolic content (TPC)

The total phenolic content in the methanolic extract was determined with Folin-ciocalteau reagent using the method of Chen *et al.* (2013)[18]. For this 1000 µg/mL gallic acid stock solution was prepared by dissolving gallic acid in methanol. 1 mL of gallic acid solution was mixed with 5.0 mL of 50 % Folin-ciocalteu reagent. After 5 min, 4.0 mL of 7.5 % Sodium Carbonate (Na_2CO_3) aqueous solution was added to the mixture, was shaken and then incubated at room tempreture for 30 min in dark. The absorbance of all samples was measured at 765 nm spectrometrically. The same procedure was repeated with the methanolic extract of the *Urginea indica*. Gallic acid was used to prepare a standard curve by preparing 1 mL aliquots of 1.0, 2.5, 5.0, 10, 15, 20, 25, 50, 100 µg/mL of gallic acid solutions ($y = 0.008x+0.018$; $r^2=0.992$, where 'y' represents the absorbance, and 'x', the concentration). The result was expressed in milligrams, Gallic acid equivalents per gram of extract (mg GAE/g extract).

Total phenolic compound extract was determined by applying the following equation:

$C= C_1 \times V/m$

Where; C=Total content of phenolic compound in mg/g, in GAE (Gallic acid equivalent),

C_1= Concentration of Gallic acid established from the clibration curve in mg/mL,

V= Volume of extract in mL,

m= Weight of plant extract in gm.

Determination of total flavonoid content

For estimation of total flavonoid content, aluminium chloride method was used[19]. In this method quercetin was used as standard and flavonoid content were measured as quercetin equivalent. For this purpose, the calibration curve of quercetin was drawn. 1mL aliquots of quercetin (1, 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600 µg/mL) was taken into 10 mL volumetric flask, containing 4 mL of distill water, 0.3 mL of 5 % $NaNO_2$ added to the flask. After 5 min, 0.3 mL 10 % $AlCl_3$ was added and volume made up to 10 mL with distill water and same process was done with the plant extract. The absorbance was noted at 510 nm using UV-visible spectrometer.

Determination of total tannins content

The tannins were determined by Folin - ciocalteu method. In 0.1 g of dry plant sample, 50 mL of water was added, boiled for 30 min and filtered. The filtrate was collected in 500 mL flask and water was added uptill the mark then to 0.5 mL aliquots 1 mL 1 % $K_3Fe(CN)_6$. 1 mL 1 % $FeCl_3$ was added. The solution volume was made 10 mL by adding water and this was left for 5 min and measured at 720 nm in an UV/Visible spectrophotometer. The actual tannin concentration was calculated on the basis of the optical absorbance values obtained for the standard solutions calibration curve in term of mg of GAE/g of plant sample.

Determination of total saponin content

Two gram of plant sample was mixed with 20 mL of 20 % aqueous ethanol and heated over water bath (55 °C) for 4 h with continuous stirring. Then the mixture was filtered and residue was re-extracted with another 20 mL 20 % ethanol. This extract was reduced to 4 mL over water bath at 90 °C and transferred to 250 mL separatory funnel. To this 10 mL of diethyl ether [$(C_2H_5)_2O$] was added and shaken vigorously. Then the aqueous layer was

recovered and 6 mL n-butanol was added. This extract was washed twice with 10 mL of 5 % aqueous sodium chloride (NaCl) and heated on water bath for evaporation. Samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Determination of total alkoloids content

For estimation of alkaloids, 5 g plant sample was mixed with 20 mL, 10 % acetic acid in ethanol, incubated for 4 h and filtered. The filtrate was kept on water bath to make it concentrated or to make its volume 1/4th the original volume. To this, drop by drop concentrated ammonium hydroxide was added to precipitate alkaloid. This solution was left to settle and the precipitate was collected in a filter paper. The collected precipitate was washed with dilute ammonium hydroxide solution and dried in oven at 40°C, until a constant weight was obtained. Then alkaloid precipitate was calculated in mg/g of the dried plant material[20].

Determination of antioxidant activity through DPPH radical scavenging activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay gives an account on the free radical scavenging ability[21]. About 1 mL of DPPH solution (0.1 mmol/L) prepared in methanol was added to 3 mL of test or standard (gallic acid) solution at different concentration (1-64 µg/mL). The mixture was incubated in dark at 30 °C for 30 min and the absorbance was measured at 517 nm and percentage inhibition was calculated. A control reaction was carried out without the test sample. Control was also carried out to determine the absorbance of DPPH, before interacting with the extract. The percentage of inhibition of extract was calculated using the following equation:

$$DPPH\ scavenging\ activity\ (\%) = \frac{AB - AA}{AB} \times 100$$

Where, AB = absorbance of DPPH in MeOH;
AA = Sample=absorbance of DPPH+ sample extract or standard in MeOH.

Results

Table 1 represents the preliminary phytochemical screening test result of significant secondary metabolities. This reveals the presence of phenol, flavonoids, tannins, saponin and alkaloids in the methanolic extract of *U. indica*.

Quantitative estimation of significant secondary metabolites, phenol and tannins has been done by comparing with standard curve of gallic acid is present in Fig. 1 and for flavonoid estimation, standard curve, quercetin is present in Fig. 2. Tables 2 & 3 show the variation of mean absorbance with concentration of gallic acid and quercetin acid respectively. Table 4 shows the contents of total phenols that were measured by Folin-ciocalteu reagent in terms of gallic acid equivalent. The result of total phenol content was calculated from the regression equation of the standard plot ($y = 0.008x+0.018$, $R^2 = 0.992$, Fig. 1). The phenol content in 1 g methanolic extract of *Urginea indica* was 6.27 mg GAE/g (gallic acid equivalent).

Table 1 — Phytochemical screening of *Urginea indica*

Phytochemical	Observation	Present/Absent
Tannin	Brownish black precipitation	Present
Saponin	Foam formed	Present
Flavonoid	Yellow color	Present
Phenol	Reddish black	Present
Alkaloid	Yellow precipitation	Present

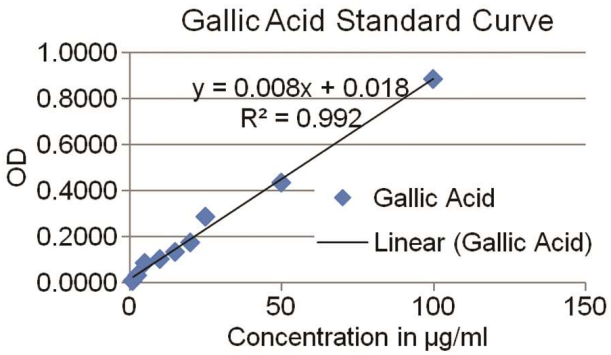

Fig. 1 — Calibration curve of standard gallic acid for determination of total phenolic and tannin content in *Urginea indica*

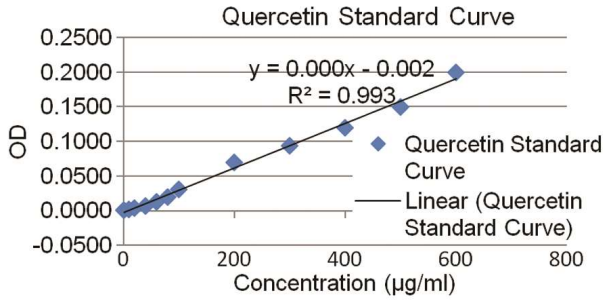

Fig. 2 — Calibration curve of standard quercetin for determination of total flavonoid content in *Urginea indica*

The total flavonoid content was calculated from the regression equation of the standard plot (y = 0.000x-0.002, R^2 = 0.993) and is expressed as quercetin equivalents (QE, Fig. 2). Total flavonoid recorded at 2 mg/mL methanolic plant extract was 13.66 mg/g quercetin equivalents (Table 4).

The tannin content was examined in plant extracts using the Folin-ciocalteu's reagent is expressed in terms of gallic acid equivalent from regression equation of the standard plot (y=0.008x+0.018, R^2=0.992, Fig. 1). The values obtained for the concentration of tannin contents are expressed as mg of GAE/g of extract. The tannin content in 0.1 g crude powder of sample plant was 130.10 mg GAE/g (Table 4).

Table 2 — Absorbance of Gallic acid in 765nm wavelength

Concentration (µg/mL)	Absorbance (Mean) λ_{max} =765 nm
1	0.008
2.5	0.0320
5	0.0860
10	0.1030
15	0.1340
20	0.1750
25	0.2870
50	0.4350
100	0.8850

Table 3 — Absorbance of standard compound (Quercetin acid)

Concentration (µg/mL)	Absorbance (Mean) λ_{max}=510 nm
1	0.0010
10	0.0020
20	0.0040
40	0.0070
60	0.0130
80	0.0200
100	0.0310
200	0.0700
300	0.0940
400	0.1200
500	0.1500
600	0.2000

Table 4 — Total phenolic, total flavonoid, tannin, saponin and alkaloid content present in methanolic extracts of *U. indica*

Parameters	Unites	Methanol extract
Total phenolic content	mg of GAE/g of extract	006.27
Total flavonoid content	mg of QE/g of extract	013.66
Tannins content	mg of GAE/g of extract	130.10
Saponins content	mg/gm of dry material	004.00
Alkoloids content	mg/gm of dry material	017.80

Saponin and alkaloid per gm dry sample of bulb of *Urginea indica* plant was found to be 4 mg and 17.8 mg, respectively (Table 4).

In the present study DPPH radical scavenging of *U. indica* extract was investigated and results were shown in Table 5. The extract showed a dose dependent scavenging activity. However, the scavenging activity of gallic acid used as standard was showed the highest activity. The IC_{50} value of methanolic extract of sample plant is calculated as 51.87 µg/mL (y = 28.49 ln (x) - 62.50, R^2 = 0.995), which is very near to the IC_{50} value of gallic acid is 39.91 µg/mL (y = 26.40 ln (x) - 47.33, R^2 = 0.988). Fig. 3 shows the comparative graph of per cent scavenging activity of plant extract with standard gallic acid.

Discussion

The secondary metabolites of various plants have been traditionally utilized for the betterment of human health[22]. In the form of diet and herbal medicines, the secondary metabolites are under intensive investigation for their potential use as chemopreventive agents to block and suppress carcinogenesis[23]. Phenolic compounds

Table 5 — DPPH radical scavenging activity of *U. indica* bulb

Concentration (µg/mL)	Per cent radical scavenging activity	
	Plant extract of *U. indica*	Gallic acid (standard)
20	22.38	32.24
40	41.63	46.38
60	54.38	63.81
80	63.05	69.64
100	71.19	74.38
200	86.48	91.41
IC_{50}	51.87	39.91

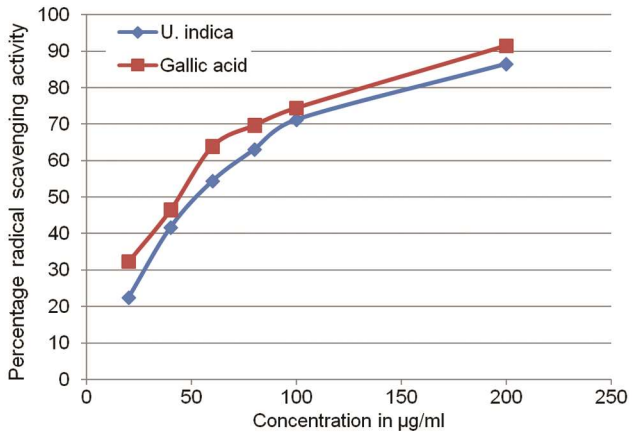

Fig. 3 — A comparative graph of per cent radical scavenging activity in *U. indica* with gallic acid

in plants play the key role as primary antioxidants or free radical scavengers. The antioxidant activity of the phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxide[24]. It has also been proposed that polyphenolic compounds provide antimutagenic and anticarcinogenic properties in humans, when ~1.0 g was consumed daily from a diet rich in vegetables and fruits[25]. Flavonoids are the most common and widely distributed group of plant's polyphenolic compounds, characterized by a benzo-γ-pyrone structure. It is ubiquitous in fruits and vegetables. Polyphenolic compounds are also believed to have chemopreventive and suppressive activities against cancer cells by inhibition of metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle[26]. Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes[27]. Saponins have been implicated as bioactive antibacterial agents of plants, which are a glycoside, have the property of precipitating and coagulating red blood cells, which are occurring widely in plants[28]. Alkaloids are the largest group of phytochemicals causing toxicity against cells of foreign organisms; they are also helpful in fighting with cancer[29]. The phytochemical studies have shown that plants with antimicrobial activity mainly contain bioactive constituents such as alkaloids, flavonoids, tannins and saponins[30]. In the present study, methanolic extract of *U. indica* demonstrated the presence of significant secondary metabolites, phenol, flavonoids, tannins, saponins and alkaloids in a good quantity.

When it comes to antioxidant activity of the *U. indica*, high DPPH radical scavenging ability was observed. DPPH has been widely used to evaluate the antioxidant activity of plant extracts. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH[31]. It undergoes reduction by an antioxidant is measured in terms of decrease in its absorbency at 517 nm. As DPPH radical reacts with a suitable reducing agent, the electron becomes paired and the solution changes from purple to yellow color. Methanolic extracts of *Urginea indica* bulbs have significant level of antioxidant activity due to the presence of bioactive compounds and can be a potential source of new useful drug[32].

Traditional significance of the study

Medicinal plants have a long tradition of use outside of conventional medicine. The medicinal plants contain so many chemical compounds which are the major source of therapeutic agents to cure human diseases. Recent discovery and advancement in medicinal and aromatic plants have led to believe that plant and plant products may be highly beneficial for health care of mankind. In some cases, improper knowledge of traditional medication practices due to lack of documentation of traditional knowledge herbal remedies may precipitate manifestation of toxicity or in the other extreme, therapeutic failure. Hence, the proper knowledge to built a scientific approach is necessary for the use of herbal medicine. The present study was undertaken to gives a scientific approach and an authentication to traditional medicinal practices of *Urginea indica.*

Conclusion

The *Urginea indica* is widely used in traditional system of medicines for the treatment of different kinds of ailments, such as cancer, cardiac problem, hypertension, dyspepsia, arterio-sclerosis, asthma, rheumatism, cold and cough, etc. In conclusion, the present study has shown that the medicinal properties of the species have been attributed by the richness of the secondary metabolites, like phenol, tannins, flavonoids, saponins and alkaloids. Phenol and flavonoids are the major contributors of antioxidant activity in *Urginea indica.* DPPH assay gave comparable result for antioxidant activity measured in methanolic extract of *Urginea* bulb. Hence, it is possible to conclude that methanolic extracts of *Urginea indica* bulbs have significant level of antioxidant activity due to the presence of bioactive compounds and can be a potential source of new useful drug. However, the plant species is a promising candidate for further investigations of bioactive compounds and isolation of a new drug. The plant species needs immediate protection, propagation and conservation as it is under threatened category.

Acknowledgement

The authors express sincere thanks to Head of the Department, Department of Botany, Ranchi University, Ranchi, for providing facilities to carry-out this work and are also thankful to Research supervisor for their guidance and counseling. Author is also thankful to BAU, Ranchi, for providing sample species.

References

1 Teklehaymanot T, Ethanobotanical study of knowledge and medicinal plants use by the people in Dek Island in Ethiopia, *J Ethnopharmacol*, 124 (1) (2009) 69-78.

2 Zhang SC, *et al.*, Traditional knowledge on "*Luchai*" [*Phagmites australis* (Cav.) Trin. Ex Steud. and *Arundo donax* L.] and their dynamics through urbanization in Yangzhou area, East China, *Indian J Tradit knowle*, 15 (4) (2016) 580-586.

3 Saha MR, Sarker DD & Sen A, Ethnoveterinary practices among the tribal community of Malda district of West Bengal, India, *Indian J Tradit Knowle*, 13 (2) (2014) 359-367.

4 Naidoo Y, *et al.*, Antibacterial, antioxidant and phytochemical properties of the ethanolic extract of *Ocimum obovatum* E. Mey. Ex Benth, *Indian J Tradit Knowle*, 15 (1) (2016) 57-61.

5 Harborne JR, Introduction to ecological biochemistry, 4th edn, (London, Elsevier), 1993.

6 Muhammad Usman Amin, Muhammad Khurram, Baharullah Khattak & Khan Jafar, Antibiotic additive and synergistic action of rutin, morin and quercetin against methicillin resistant *S. aureus*, *BMC Comple Altern Med*, 15 (59) (2015) 1-12.

7 HK Hairy shaw, In: A Dictionary of Flowering Plants and Ferns, edited by JC Wills, (Cambridge University Press, Cambridge), 1993.

8 Hemadri K & Swahari S, A new species of Liliaceae from India, *Ancient Sci Life*, 2 (1982) 78–81.

9 Bozorgi Mahbubeh *et al.,* Traditional medical uses of *Drimia* species in terms of phytochemistry, pharmacology and toxicology, *J Tradit Chin Med*, 37 (1) (2017) 124-139.

10 Choudhary K, Singh M & Phillai U, Ethnobotanical Survey of Rajasthan – An Update, *American-Eurasian J Bot*, 1 (2008) 38-45.

11 Swarnkar S & Katewa SS, Ethnobotanical observation on tuberous plants from tribal area of Rajasthan (India), *Ethnobot Leaflets*, 12 (2008) 647-666.

12 Raj M Smitha, Kameshwari MN Shiva, Tharasaraswathi KJ & Shubharani R, Qualitative and Quantitative Analysis of Phytochemicals in two different species of *Urginea*, *Int J Pharm Life Sci*, 8 (2) (2017) 5433-5438.

13 Deepak AV & Salimath Bharathi P, Antiangiogenic and proapoptotic activity of a novel glycoprotein from *U. indica* is mediated by NF-kB and Caspase activated DNase in ascites tumor model, *Biochimie*, 88 (2006) 297–307.

14 Ranjan Saha Manas, Pallab Kar & Arnab Sen, Assesment of phytochemical, antioxidant and genetic diversities among selected medicinal plant species of Mimosoideae (Mimosaceae), *Indian J Tradit Knowle*, 17 (1) (2018) 132-140.

15 Jain P, Singh SK, Sharma HP & Basri F, Phytochemicals Screening and Antifungal Activity of *Semecarpus anacardium* L. (An Anti-Cancer Plant), *Int J Pharmaceut Sci Res*, 5 (5) (2014) 1884-1891.

16 Harborne JB, *Phytochemical methods*-a guide to modern techniques of plant analysis, 2nd edn, (London: Chapman and Hall), 1984.

17 Evans WC, *Trease Evans Pharmacognosy*, 14th edn, (London: WB Saunders Ltd), 1966, 119-159.

18 Chen Z, Bertin R & Froldi G, EC_{50} estimation of antioxidant activity in DPPH assay using several statistical programs, *Food Chem*, 138 (1) (2013) 414-20.

19 Sahu Rajeshwari & Saxena Jyoti, Screening of total phenolic and flavonoid content in conventional and non-conventional species of curcuma, *J Pharmaco Phytochem*, 2 (1) (2013) 176-179.

20 John B, Sulaiman CT, George S & Reddy VRK, Spectrophotometric estimation of total alkaloids in selected *Justicia* species, *Int J Pharm Pharm Sci*, 6 (5) (2014) 647-648.

21 Vani T, Rajani M, Sarkar S & Shishoo CJ, Antioxidant properties of the *Ayurvedic* formulation-*Triphala* and its constituents, *Int J Pharmacogn*, 35 (5) (1997) 313-317.

22 Choudhary Neha & Singh Vikram, A census of *P. longum's* phytochemicals and their network pharmacological evaluation for identifying novel drug-like molecules against various diseases, with a special focus on neurological disorders, *PLoS One*, 13(1) (2018) 1-31.

23 Wang Lei & Chen Chi, Emerging Applications of Metabolomics in Studying Chemopreventive Phytochemicals, *The Am Asso Pharmaceut Sci J*, 15 (4) (2013) 941–950.

24 Osawa T, Novel natural antioxidants for utilization in food and biological systems, Post harvest biochemistry of plant food-materials in the tropics, *Japan Scientific Press*, Tokyo, Japan, 1994, 241-251.

25 Tanaka M, Kuie CW, Nagashima Y & Taguchi T, Applications of antioxidative maillard reaction products from histidine and glucose to sardine products, *Nippon Suisan Gakaishi*, 54 (1998) 1409-1414.

26 DJ Newman, G M Gragg, S Holbeck & EA Saus-ville, Natural Products as Leads to Cell Cycle Pathway Targets in Cancer Chemotherapy, *Curr Cancer Drug Targets*, 2 (4) (2002) 279-308.

27 US Patil & OS Deshmukh, Preliminary phytochemical screening of six medicinal plants used as traditional medicine, *Int J Pharm Bio Sci*, 7(1) (2016) 77-81.

28 Indrayan AK, Sharma S, Durgapal D, Kumar N & Kumar M, Determination of nutritive value and analysis of mineral elements for some medicinally valuated plants from Uttaranchal, *Curr Sci*, 89 (7) (2000) 1252-1253.

29 Nobori T, Miurak K, Wu DJ, Takabayashik LN & Carson DA, Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers, *Nature*, 368 (1994) 753-756.

30 Chukwuka KS, Ikheloa JO, Okonko IO, Moody JO & Mankinde TA, Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of some medicinal plants, *Adv Appl Sci Res*, 2 (2011) 37-38.

31 M Sreejith, N Kannappan, A Santhiagu & Ajith P Mathew, Phytochemical, Anti-oxidant and Anthelmintic activities of various leaf extracts of *Flacourtia sepiaria* Roxb, *Asian Pac J Trop Biomed*, 3(12) (2013) 947-953.

32 Misra Banani, MN Shivakameshwari & R Shubarani, Antioxidant activity in two species of *Urginea steinhill*. Hyacinthaceae, *Int J Rec Sci Res*, 6 (5) (2015) 3807-3811.