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Nutritional, anti-nutritional profile and phytochemical screening of flowers of *Indigofera tinctoria* from Garhwal Himalaya

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ABSTRACT
Indigofera tinctoria flower has been found to rich in medicinal properties such as anticancer, antidibetic, antimicrobial activity. The plant flower have been found to rich in nutrients and antinutrients such as crude protein 2.63(%), carbohydrates 23.92(%), crude fibre 19.65(%), crude fat 1.0(%) and antinutrients alkaloids 0.95(%), flavonoids 2.40(%), saponins 2.56(%) and tannins 0.23(%) respectively. This analysis revealed that the plants contained potent medicinal properties as compared to another medicinal plant.

Keywords: Nutritional value, Antinutritional value, Successive value, Thin Layer Chromatography and Phytochemical Screening.

1. Introduction
Indigofera tinctoria belongs to the family of Fabaceae which is commonly known as Sakina in Uttarakhand. *Indigofera tinctoria* are used in liver disease, heart disorder and gout [1]. The roots, stems and leaves are bitter, thermogenic, trichogenous, expectorant, anthelminthic [2], cardiopathy, asthma, skin diseases, diuretic and are useful for promoting growth of hair. The plant is stimulant, deobstruent and purgative. *Indigofera tinctoria* is antiseptic and astringent. The juice of the leaves and powder are used mixed with honey in enlargement of liver & spleen, epilepsy and other nervous affections. Juice is also given in asthma, whooping cough, palpitation of heart, in some lung diseases and kidney complaints as in dropsy. The ethanolic extract of dried leaves of *Indigofera tinctoria* showed significant activity and decrease in blood glucose level of rabbits as estimated by Folin-Wu Method [3]. In this experiment, alloxan is used as diabetes inducing agent. *Indigofera tinctoria* showed that it posse's Anti-hyperglycaemic activity, Anti-bacterial, Antioxidant, cytotoxicity effect, anti-inflammatory activity, anti hepatoprotective activity, antidiabetic activity and anticonvulsive agent.

2. Materials and Methods:-
2.1 Plant Material: -
Flowers of *Indigofera tinctoria* were collected from adjoining area of Dugada village Distt-Tehri Garhwal Uttarakhand) in the month of March– April 2012. The plant was authenticated by botanist Prof. R. D. Guar, Department of Botany and the voucher specimen number is GUH 7284, deposited in Deptt of Botany, H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand, India.

Indigofera tinctoria flowers & seeds

2.2 Preparation of Plant Extract: -
The plant material were separated into its selected part flower air dried ground to moderately fine powder and soxhlet extracted with increasing polarity solvent (Petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water) [4]. Each extract were evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of flower were subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 500 gm). The various concentrated extracts were stored in air tight container for further studies.

2.3 Nutritional, Anti-nutritional value & Minerals assay: -
The edible portion of flower was analyzed for moisture, ash, fat [5] and fiber as per method reported in AOAC. Total nitrogen was analyzed by micro-kjeldhal method [6] and for crude protein the value was multiplied by 6.25. Total carbohydrates were obtained by subtracting the value moisture, crude protein, crude fat, crude fiber and ash from 100% [7]. The total energy value equal to addition of fat, protein and sugars calorie, each gram of fat give 9 kcal, protein and sugar give 4 kcal energy. The minerals analyzed were Potassium using atomic absorption spectrophotometer, calcium and phosphorus by flame photometer. Ascorbic acid in flower was estimated by standard process [8].

2.4 Successive Value: -
Accurately weighed 500 gm coarse and air dried drug material were subjected to hot successive continuous extraction in soxhlet apparatus with different solvents with increase in polarity petroleum ether, benzene, chloroform, methanol, ethanol and finally with water. The extracts were filtered in each step concentrated and the solvent was removed by vacuum distillation. The extracts were dried in the vacuum desiccators and the residues were weighed [9]. Which contain maximum chemical compound are these categories as depend upon solvent nature and types.

2.5 Detection of Chemical Compound through TLC:-
Thin layer chromatography (TLC) is a chromatography technique used to separate unknown chemical compound mixtures. Thin layer chromatography is performed on a sheet of glass, which is coated with a thin layer of adsorbent material usually silica gel G. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Thin Layer Chromatographic plates are prepared by spreading silica gel G on glass plate using distilled water as solvent these plates are activated in oven at 110 OC for 1 hour. All six extracts are applied separately and run in different solvent system of varying polarity. These plates are developed in UV-Visible chamber, Iodine chamber and spraying reagent for different spots and R_f value of chemical constituent [10].

2.6 Phytochemical Analysis:-
Preliminary phytochemical analysis of all extract were prepared by weighing and the dried powdered flower were subjected to hot successive continuous extraction with different solvents as per the polarity petroleum ether, benzene, chloroform, methanol, ethanol and finally with water. The extracts were filtered in each step concentrated and the solvent was removed by vacuum distillation. The extracts were dried over desiccators and the residues were weighed. The presence or absences of the primary and secondary phytoconstituents were detected by using standards methods [11].

3. Result and discussion:-
Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the nutritional value & anti-nutritional value, TLC analysis, successive extraction and phytochemical screening. The results of nutritional value & anti-nutritional value, mineral value, TLC analysis, successive extraction and phytochemical screening as table 1, 2, 3, 4 and 5.

3.1 Nutritional Value: -
The level of nutrients such as crude protein, carbohydrates, crude fiber and ash content (2.63%, 23.92%, 19.65% and 5.20%) and also minerals as calcium, magnesium, potassium and phosphorus (9.38, 3.25, 1.61 and 0.25 mg/100gm) respectively.

Table 1: Nutritional value and antinutritional value of *Indigofera tinctoria* flower

Nutrients	Value	Nutrients	Value
Moisture (%)	47.60±0.10	Insoluble ash (%)	8.54 ± 0.10
Ash (%)	5.20± 0.15	Soluble ash (%)	91.46 ± 0.10
Crude fat (%)	1.00±0.20	Insoluble acid (%)	93.2±0.05
Crude fibre (%)	19.65±0.14	Soluble acid (%)	6.80±0.10
Total nitrogen (%)	0.42±0.05	Insoluble base (%)	50.9±0.15
Total protein (%)	2.63 ± 0.08	Soluble base (%)	49.1±0.08
Carbohydrate (%)	23.92± 0.10	Water insoluble (%)	86.7±0.10
Organic matter (%)	94.80± 0.15	Water soluble (%)	13.3±0.05
Total saponins (%)	2.56±0.40	Total phenolic (%)	2.63±0.05
Total flavonoid (%)	2.40±0.50	Total tannins (%)	0.23±0.25
Total alkaloid (%)	0.95±0.10		

Table 2: Mineral value of *Indigofera tinctoria* flower

Mineral	Value(gm/100gm)	Mineral	Value(gm/100gm)
Zn	0.13	Fe	0.32
Pb	0.03	Cr	0.02
Cu	0.03	Mn	0.05
Co	0.01	Ca	9.38
P	0.25	Mg	3.25
K	1.61	Sr	0.11

3.2 Successive Value: -

Indigofera tinctoria flower contains significant value 61.54%, 22.30% and 15.23% against ethanolic, water and methanolic solvent extract with 500 gm plant sample.

Table 3: Observations of thin layer chromatographic (TLC) studies of flower of *Indigofera tinctoria*, W: C: M (Water: Chloroform: Methanol, 10:64:28-36)

Extract	Mobile phase	No. of spot	R_f Value	hR_f Value
Pet. Ether Extract	(C:M:W) 64:30:10	1	(0.16)	(16)
Benzene Extract	(C:M:W) 64:28:10	1	(0.16)	(16)
Chloroform Extract	(C:M:W) 64:26:10 64:28:10 64:30:10	2 1 2	(0.85, 0.93) (0.64) (0.85, 0.93)	(85, 93) (64) (85, 93)
Methanolic Extract	(C:M:W) 64:26:10 64:28:10 64:30:10	2 3 5	(0.32, 0.78) (0.16, 0.64, 0.78) (0.16, 0.32, 0.64, 0.78, 0.93)	(32, 78) (16, 64, 78) (16, 32, 64, 78, 93)
Ethanolic Extract	(C:M:W) 64:26:10 64:28:10 64:30:10	3 3 5	(0.16, 0.32, 0.78) (0.16, 0.64, 0.78) (0.16, 0.32, 0.64, 0.78, 0.93)	(16, 32, 78) (16, 64, 78) (16, 32, 64, 78, 93)
Water Extract	(C:M:W) 64:30:10	1	(0.78)	(78)

Table 4: Extractive values of *Indigofera tinctoria* flower

Method of extraction	Values of three replicates (%w/w)	Mean (% w/w) ± SEM
Cold maceration:		
1) Water soluble	(10.90,10.10& 10.80)	(10.26±0.20)
2) Alcohol soluble	(19.10,20.20& 20.50)	(19.93±0.12)
Hot Extraction:		
1) Pet. Ether soluble	(2.20,1.90&2.10)	(2.06±0.05)
2) Benzene soluble	(1.40, 1.96 & 2.10)	2.15 ± 0.20
3) Chloroform soluble	(2.90,2.60&2.80)	(2.76±0.34)
4) Methanol soluble	(15.80,14.80&15.10)	(15.23±0.50)
5) Ethanol soluble	(60.92, 61.57 & 62.13)	61.54 ± 0.85
6) Water soluble	(21.90,22.80&22.20)	(22.30±0.90)

Table 5: Phytochemical screening of *Indigofera tinctoria* plant flower, (+) – Present, (-) – Absent

Test	Pt. ether Extract	Benzene Extract	Chloroform Extract	Methanolic Extract	Ethanolic Extract	Water Extract
Carbohydrates/glycosides						
(1) Molish test	(-)	(-)	(-)	(+)	(+)	(+)
(2) Fehling test	(-)	(-)	(-)	(+)	(+)	(+)
(3) Benedict test	(-)	(-)	(-)	(+)	(+)	(+)
Alkaloid						
(1) Mayer's test	(-)	(-)	(-)	(-)	(-)	(-)
(2) Dragondroff test	(-)	(-)	(-)	(-)	(-)	(-)
Flavonoids						
(1) Shinoda/pew	(-)	(-)	(-)	(+)	(+)	(+)
(2) Ammonia	(-)	(-)	(-)	(+)	(+)	(+)
Saponins	(-)	(-)	(-)	(-)	(+)	(+)
Tannins						
(1) Pyrogoll & catechol	(-)	(-)	(-)	(+)	(+)	(-)
(2) Gallic acid	(-)	(-)	(-)	(+)	(+)	(-)
Unsaturated sterol/triterpenes						
(1) Liebermann Burchard test	(-)	(-)	(-)	(-)	(+)	(-)
(2) Salkowiskis test	(-)	(-)	(-)	(-)	(+)	(-)
Resin	(-)	(-)	(-)	(+)	(+)	(-)
Phenolics compound						
(1) Ferric chloride	(-)	(-)	(-)	(+)	(+)	(-)
(2) Nitric acid	(-)	(-)	(-)	(+)	(+)	(+)
Protein and amino acid						
(1) Xanthoprotien	(-)	(-)	(-)	(+)	(+)	(+)

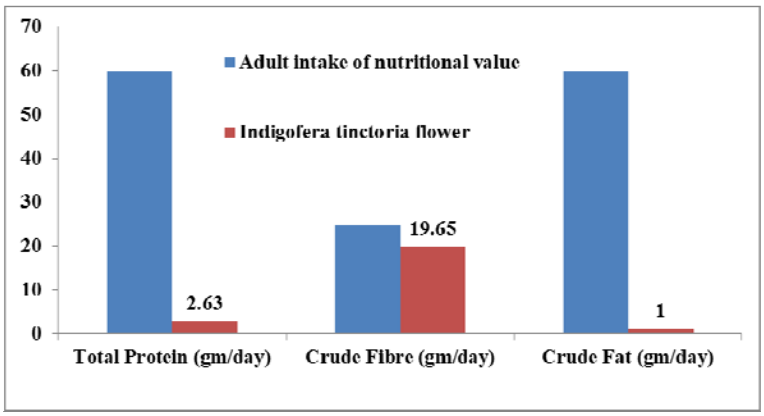

Fig 1.1: Comparison of per day intake of nutrients by Adults with the nutrients present in the flower of *Indigofera tinctoria*

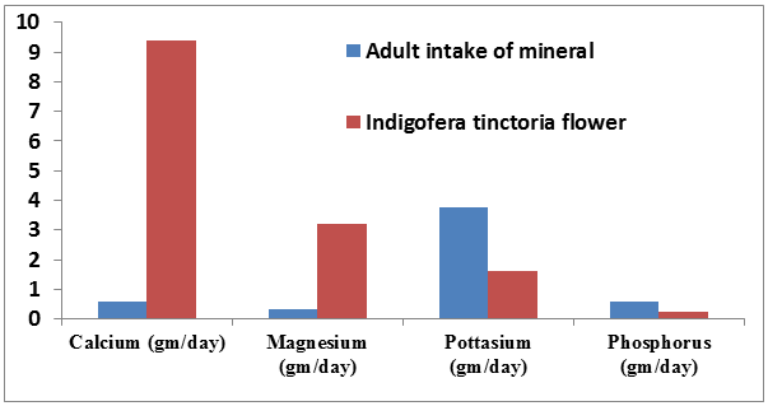

Fig 2.1: Comparison of per day intake of minerals by Adults with the mineral present in the flower of *Indigofera tinctoria*

Fig 3.1: Thin layer chromatography qualitative analyses of six fractions against *Indigofera tinctoria* plant flower extract

3.3 Phytochemical Screening:-

The phytochemical screening of plant for the presence of glycosides, flavonoids, phenols, resin and tannins. This analysis revealed that the flower contained higher value of fat, protein, fiber and minerals as compared to the cultivated flowers with rose and 250 gm flowers contain sufficient amount of nutrients required per day by a person.

4. Conclusion:-

The flower of *Indigofera tinctoria* contain phytoconstituents like alkaloids, steroids, fats & fixed oil, flavonoids, tannins, proteins and carbohydrates. The TLC results of the ethanolic, methanol and water extract show that at least six different phytoconstituents were present in each extract of *Indigofera tinctoria* flower. More detailed study must be done for farther isolation leading to the pure compounds.

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