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Preliminary pharmacognostic and chemical test, HPTLC, study of Stevia Bertoni (Honey Leaf)

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Abstract
Stevia rebaudiana Bertoni (Bert.) is a supplement rich regular option in contrast to counterfeit sugar, has a place with Asteraceae family and contains more than hundred phytochemicals. The main parts of S. rebaudiana is steviol glycosides, and that gratitude to its high power improving which assessed around multiple times better than saccharose however alongside this has no calorific worth. Many exploration exercises on its biochemical and organic properties have been done in late past. Likewise, stevia has various remedial qualities in the treatment of patients with diabet-related corpulence, hypertension or heart infection, cancer prevention agent, antimicrobial and antifungal action, for which improving properties have been recognized. The point of this survey is to introduce biochemical organization, healthy benefit and utilization of stevia leaves and its helpful worth to wellbeing.

Keywords: stevia rebaudiana, asteraceae, biochemical, natural properties, sugar, wholesome

Introduction
Stevia rebaudiana Bertoni is an extended ragged bush of the Asteraceae family, local to the Amambay district in the north east of Paraguay. It additionally happens in the adjoining portions of Brazil and Argentina (Soejarto, 2002). Today its development has spread to different districts of the world, including Canada and a few pieces of Asia and Europe (Amzad-Hossain et al., 2010) [5]. Among the 230 species in the family Stevia, just the species rebaudiana and phlebophylla produce steviol glycosides (Brandle et Telmer, 2007) [12]. Stevia rebaudiana was organically characterized in 1899 by Moisés Santiago Bertoni, who depicted it in more detail. At first called Eupatorium rebaudianum, its name changed to S. rebaudiana Bertoni in 1905.
The sweet rule was first secluded in 1909 and just in 1931 was the concentrate filtered to create stevioside, the synthetic design of which was set up in 1952 as a diterpene glycoside.

Classification of Stevia Rebaudiana Plant Is
Realm: Plantae

Subkingdom: Tracheobionta

Superdivisio: Spermatophyta

Divisio: Magnoliophyta

Class: Magnoliopsida

Sub-class: Asteridae

Ordo: Asterales

Familia: Asteraceae

Class: Stevia Cav

Fig 1: Stevia rebaudiana Bertoni created in Morocco

Stevioside is portrayed as a glycoside containing three glucose particles connected to an aglycone, the steviol moiety. During the 1970s, different mixtures were segregated, including rebaudioside A, with an improving strength considerably higher than stevioside S. rebaudiana has drawn in financial and logical interests because of the pleasantness and the alleged helpful properties of its leaf. Japan was the primary country in Asia to showcase stevioside as a sugar in the food and medication industry. From that point forward, the development of this plant has extended to different nations in Asia, including China, Malaysia, Singapore, South Korea, Taiwan, and Thailand (Chatsudthipong and Muanprasat, 2009) [15]. Stevia and stevioside have been applied alternative for saccharose, for treatment of diabetes mellitus, weight, hypertension, and caries anticipation, and various investigations have proposed that, other than pleasantness, stevioside, alongside related mixtures which incorporate rebaudioside A, steviol, and is steviol, may likewise offer helpful advantages, as they have

against hyperglycemic, antihypertensive, calming, hostile to the tumor, hostile to diarrhoeal, diuretic, and immunomodulatory impacts The leaves of stevia have useful and tangible properties better than those of numerous other high-intensity sugars and are probably going to turn into a significant wellspring of high-strength sugar for the developing common food market later on Toxicological examinations have shown that stevioside doesn't have mutagenic, teratogenic, or cancer-causing impacts. Moreover, unfavorably susceptible responses have not been seen when it is utilized as a sweetener.

The reason for this audit is to choice of fundamental data coming from a few logical explores on stevia, a normally bio-sugar. significance was set on the astounding capability of stevia as an exceptional high-strength sugar with its nourishing, remedial, practical properties and wellbeing advancing properties.

Organic depiction

Stevia is a class of around 200 types of spices and bushes in the sunflower family (Asteraceae). It grows up to 1 m tall (Mishra et al., 2010), 60 cm to 1m tall (Serio, 2010). The plant is a perpetual spice with a broad root framework and fragile stems creating little, elliptic leaves (Shock, 1982). The leaves are sessile, 3-4 cm long, extend lanceolate or spatulate formed with gruff tipped lamina, the serrate edge from the center to the tip, and whole beneath. The upper surface of the leaf is somewhat granular pubescent. The stem is woody and feeble pubescent at the base. The rhizome has somewhat expanding roots. The blossoms are pentamerous, little and white with a pale purple throat. They are compositely encircled by an involucre of epicalyx. The capitula are in free, sporadic, sympodial cymes. The minuscule white florets are borne in little corymbs of 2-6 florets, orchestrated in free panicles. The organic product is a five ribbed axle molded achene (Lemus-Modaca et al., 2012). Stevia will develop well on a wide scope of soils given a predictable inventory of dampness and satisfactory seepage; plants under development can reach up to 1 m or more in stature (Shock, 1982). It is developed as an enduring bush in subtropical districts including portions of the United States. The plant is native toward the northern locales of South America and fills wild in the Highlands of Amambay and close to the wellspring of the stream Monday (a boundary region among Brazil and Paraguay). It is being developed in mainland China, Taiwan, Thailand, Korea, Brazil, and Malaysia. Other than the previously mentioned nations, stevia is likewise filled in Israel, Ukraine, the UK, the Philippines, Canada, Hawaii, California, and all over South America (Sivaram and Mukundam, 2003). Stevia has an astounding water need, the leaves and stems can wither quickly, yet in addition, recuperate quickly if the pressure isn't drawn out; this is a restriction to the space appropriate for its development. It develops quickly and can be developed as a yearly spice during pre-summer and summer (Lemus-Modaca et al., 2012). Stevia can be filled in generally helpless soil. The plants can be utilized for business creation for a very long time at the stretch of which harvests of vegetative parts happen six times each year. The roots stayed set up and the plant recovers quickly. The number of dry leaves that can be gathered changes from 15 to 35 g for each plant (Mishra et al., 2010). As indicated by Serio (2010), one planted hectare can deliver somewhere in the range of 1000 and 1200 kg of dried leaves that contain 60-70 kg stevioside, which is a low yield contrasted with sugar stick or sugar beet. In any case, 70 kg stevioside, which is multiple times better than saccharose, is comparable to a yield of 21,000 kg sugar for every hectare.Biochemical and nutritional aspects of stevia.The dry concentrate from the leaves of stevia contains flavonoids, alkaloids, water-dissolvable chlorophylls and xanthophylls, hydroxycinnamic acids (caffeine, chlorogenic, and so forth), impartial water-solvent oligosaccharides, free sugars, amino acids, lipids, fundamental oils, and minor components (Komissarenko et al., 1994).Savita et al., 2004 investigated stevia leaves on a dry weight premise and determined energy worth of 2.7 kcal g-1, additionally, Khiraoui et al., 2017 [28] being examined were discovered to be (3.05 - 3.17 kcal g-1) This implies that stevia might be allowed the situation with a low-calorie sugar. Calorie commitment to the eating regimen by the ordinarily utilized saccharose, which is viewed as high since it is processed totally by the body, can possibly escalate towards overweight status. In this specific situation, the utilization of stevia as a low-calorie sugar could be of gigantic assistance in confining or controlling calorie consumption in the eating routine (Lemus-Modaca et al., 2012).As indicated by Mishra et al. (2010) stevia leaf presents upsides of the mass thickness of 0.443 g ml-1, water-holding limit of 4.7 ml g-1, fat assimilation limit of 4.5 ml g-1, emulsification worth of 5.0 ml g-1, expanding list of5.01 g-1, solvency of 0.365 g-1, and pH of 5.95. The investigation of Mishra et al. (2010) showed an expanded water holding limit of the stevia leaf powder, which gives off an impression of being beneficial and might be because of high protein content. Proteins would build a water-holding limit, in this way improving the growing capacity, a significant capacity of protein in the readiness of gooey food sources like soups, flavors, batter, and prepared items. The capacity of the protein to help the arrangement and adjustment of emulsion is likewise basic in numerous food source applications, like cake, players, espresso whiteners, milk, frozen pastries, and others. Stevia leaf powder appears to have a satisfactory fat ingestion limit, permitting it to assume a significant part in food handling since fat follows up on flavor retainers and builds the mouthfeel of food varieties. The advantages related to stevia leaf are basically because of their biochemical and healthful creation which is a decent wellspring of starches, protein, and rough fiber that advances wellbeing and limit the danger of specific illnesses. Carbs Sugars are the chief wellsprings of energy and they are found as primary segments of cell components (Lemus-Modaca et al., 2012). The benefits related to stevia leaf are basically because of their healthful organization (Table 1), which is a decent wellspring of sugars. Starches play out various fundamental parts in living creatures. Subsequently, monosaccharides are the significant wellspring of energy in human digestion, while polysaccharides fill in as the capacity of energy and can go about as primary parts. Other useful wellbeing impacts have likewise been connected to these mixtures. This incorporates a prebiotic impact just as other more uncommon cell reinforcement or calming exercises (Bernal et al., 2011) [9]. In S. rebaudiana roots and leaves, inulin-type fructooligosaccharides at 4.6%, a normally happening plant polysaccharide with significant useful properties identified with prebiotics, dietary fiber, job lipid digestion, and diabetes control, have been separated by Braz de Oliveira et al. (2011) [13].

Proteins

Proteins, peptides, and additional amino acids are found in an incredible assortment of networks including creatures, parasites, vegetables, cereals, and so on (Bernal et al., 2011) [9]. The investigation of Mohammad et al., (2007) distinguished eight fundamental amino acids in stevia leaves, specifically glutamic corrosive, aspartic corrosive, lysine, serine, isoleucine, alanine, proline, tyrosine. Abou-Arab et al. (2010) [1] and Li et al. (2011) had discovered even more amino acids in the stevia leaves. Inside and out seventeen amino acids were resolved and delegated fundamental and superfluous amino acids, shockingly including arginine as one of the crucial amino acids.

Rough fiber

Dietary fiber is the food parts of plants or closely resembling carbs that are impervious to assimilation and retention in the human small digestive tract with complete or halfway aging in the internal organ (Sankhala et al., 2005). Dietary fiber incorporates polysaccharides, oligosaccharides, lignin, and related plant substances. Moreover, a comparable to sugar is characterized as those carbs-based food fixings that are non-edible and non-absorbable, and which are like plant dietary fiber (Prosky, 2001). Dietary fiber has been generally read for its medical advantages. It is viewed as a preventive factor for disease, fills in as a substrate for colonic microscopic organisms, advances intestinal food travel, and diminishes bile corrosive reabsorption consequently changing micelle development and adding to bringing down blood cholesterol levels (Escudero and González, 2006) [19].

Minerals

Minerals have numerous significant capacities in the human body. The primary components are sodium, magnesium, phosphorus, sulfur, chlorine, potassium, and calcium which are named macronutrients, and the minor components, thought about micronutrients, are chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum, and iodine (Adoteyet al., 2009). Stevia contains generous measures of these significant supplements, which further sets up it as a mineral stacked fixing expected to secure the body, control, and keep up the different metabolic cycles. Potassium, calcium, magnesium, and sodium which are healthfully significant, were found insensible sum in stevia leaves (Choudhary and Bandyopadhyay, 1999) [16]. As detailed by certain creators, the mean groupings of full scale and miniature components that have been resolved in dried stevia leaves are. Lipids are a huge gathering of common mixtures. Their fundamental organic capacities incorporate energy stockpiling (Bernal et al., 2011) [9]. In the leaf oil of stevia, Tadhani and Subhash (2006a) and Atteh et al. (2011) [7] had distinguished six unsaturated fats utilizing methyl ester forms. Palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids were recognized in the leaf oil.

Vitamins

Kim et al. (2011) [29] contemplated the measures of water-dissolvable nutrients in the stevia leaf removes (Table 5), and confirmed that the substance of folic corrosive, nutrient C and nutrient B2 in the leaf separates were fundamentally higher than those of the callus extricates. In the leaf extricate, folic corrosive was discovered to be the significant compound, trailed by nutrient C. In the callus extricate, nutrient C was the significant compound, trailed by nutrient B.

Phytochemical constituents

Therapeutic plants are vital to the strength of people and networks. The restorative worth of these plants lies in some synthetic substances that produce a positive physiological activity on the human body (Edeoga et al., 2005) [18]. The phytochemicals present in S. rebaudiana are austroinullin, β-carotene, glucoside, niacin, rebounds oxides, riboflavin, steviol, stevioside, and thiamine (Jayaraman et al., 2008) [20].

Diterpene glycosides

Glycosides are a gathering of natural mixtures containing a starch particle (sugar) bound to a non-carb moiety. These mixtures are for the most part found in plants, and they can be changed over, by hydrolytic cleavage, into a sugar and a non-sugar segment (aglycone) (Bernal et al., 2011) [9]. Stevia, the regular name for the concentrate stevioside from the leaves of S. rebaudiana, is another promising inexhaustible crude foodstuff on the world market and is a characteristic, sweet-tasting calorie-free organic that may likewise be utilized as a sugar substitute or as an option in contrast to fake sugars (Anton et al., 2010) [6]. The normal sugars of stevia leaves, called steviol glycosides, are diterpenes, secluded and distinguished as stevioside, steviolbioside, rebaudioside A, B, C, D, E, F, and glucoside (Geuns, 2003) [24].

Contingent upon development conditions, development, and culturing procedures their substance range from 4 to 20% new leaf weight (Pól et al., 2007). Stevioside was accounted for to be the most plentiful stevia glycoside (4-13% w/w) found in the plant leaves. It is trailed by rebaudioside A (2-4% w/w), rebaudioside C (1-2% w/w) and dulcoside A (0.4-0.7% w/w) (Makapugay et al, 1984). Steviolbioside, rebaudioside B, D, E, and F were additionally recognized in the leaf extricates, however as minor constituents (Geuns, 2003) [24].

As indicated by Mishra et al. (2010) the stevioside is the principle improving compound found in the leaf of plant Stevia rebaudiana (from 5-15% dry weight), trailed by rebaudioside (3-6%). The stevioside and the other stevia glycosides have high compound strength in light of their tridimensional synthetic structure which produces protection from corrosive and enzymatic hydrolysis guaranteeing their inalterability much under biochemical and physiological viewpoints. Stevia appears ND, not decided All diterpene glycosides disengaged from S. rebaudiana leaves have the equivalent steviol spine (Fig. 2) and contrast predominantly in the substance of starch deposits (R1 and R2), mono-, di-, and trisaccharides containing glucose and additionally rhamnose at positions C13and C19

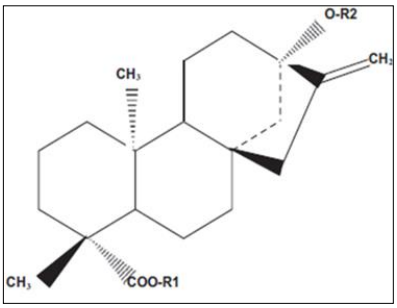

Fig 2

The pleasantness of any of the stevia compounds is more noteworthy than that of saccharose;
rebaudioside A (250-450 times); rebaudioside B (300-350 times); rebaudioside C (50-120 times); rebaudioside D (250-450 times); rebaudioside E (150-300 times); dulcoside A (50-120 times); and steviolbioside (100–125 times). Overall, the pleasantness of the steviol glycosides is 250-300 times more noteworthy than that of saccharose, with low water solvency and high liquefying focuses (Crammer et Ikan, 1987) [17]. Stevioside, the most bountiful steviol glycoside in the leaf of the plant, has gotten notable for its extreme pleasantness (250-300 times better than arrangements containing 0.4% saccharose) and is utilized as a non-caloric sugar in a few nations (Gardana et al., 2010) [22]. Furthermore, look at stevia leaf powder and stevia white concentrate with granulated sugar.

Cell Reinforcements Movement
Dynamic oxygen-free revolutionaries have been ensnared as causative specialists of malignant growth, atherosclerosis, cerebral and heart ischemia, Parkinson's illness, gastrointestinal aggravations, and maturing (Ames et al., 1993) [4]. Numerous homegrown and some basic restorative plants are acceptable wellsprings of cancer prevention agent compounds, including phenolic compounds (flavonoid, phenolics) are known to have potential cell reinforcement properties (Larson, 1988). In addition, stevia leaves have a high measure of phenolic compounds, nutrient C, carotenoids, chlorophylls Stevia leaf extricate displays a serious level of cancer prevention agent action. The cell reinforcement action of stevia leaf removal has been ascribed to the rummaging of free extreme electrons and superoxides (Thomas and Glade, 2010). A new report evaluating the in vitro capability of ethanolic leaf concentrate of S. rebaudiana demonstrates that it has a huge potential for use as a characteristic cell reinforcement (Shukla et al., 2009). Leaves of S. rebaudiana were found to contain polyphenolic compounds showing cancer prevention agent properties

Different constituents
The presence of naturally significant optional plant items in stevia leaf adds to its therapeutic worth since they show physiological action (Sofowara, 1993). These auxiliary plant constituents incorporate labdanes, flavonoids, sterols, triterpenoids, chlorophylls, natural acids, mono-disaccharides, and inorganic salts (Gardana et al., 2010) [22]. Savita et al. (2004) tracked down a high level of hostility to wholesome elements in concentrates of stevia leaf broke up in the water: oxalic corrosive and tannins. Oxalic corrosive may block the bioaccessibility of calcium, iron, and different supplements as on account of green verdant vegetables. Tannins have been accounted for to have a few pharmacological exercises, for example, spasmolytic movement in smooth muscle cells (Tona et al., 1999).

Standard Advantages of Stevia
The rule benefits of stevia development are given beneath:
1. stevia is a totally common non-manufactured item: Stevioside (the sugar) contains definitely no calories;
2. The leaves can be utilized in their common state;
3. It has gigantic improving force; just little amounts should be utilized;
4. The plant is non-harmful;
5. The leaves just as the unadulterated stevioside concentrate can be cooked;
6. Stable when warmed up to 200°C;
7. Non-fermentative;
8. Flavor improving;
9. Clinically tried and often utilized by people without antagonistic impact

Medical Advantages
Many plant glycosides have shown action in malignant growth counteraction, just as antidiabetic, against stoutness, antibacterial or antineoplastic impact (Bernal et al., 2011) [9]. As toxicological investigations have appeared, that stevioside doesn't have mutagenic, teratogenic, or cancer-causing impacts and no hypersensitive response has been seen when it is utilized as a sugar. Stevia and stevioside have been applied as alternatives for sucrose, for treatment of diabetes mellitus, corpulence, hypertension, and for the avoidance of caries. Stevioside additionally shows bactericidal movement and represses the development of Escherichia coli. (Pól et al., 2007). They can likewise go about as an enemy of the cariogenic items, hostile to gum disease, and be an assistant in corpulence since it is a sugar that isn't processed, that is, it has no calories, and accordingly, it isn't stuffing (Blauth de Slavutzky, 2010) [11]. The toxicology of stevioside has been widely examined, and related information, rethought recently, showed it to be non-poisonous, non-mutagenic, and non-cancer-causing. It was additionally plainly shown that high centralizations of the sugar rebaudioside A, controlled in the eating regimen of rodents more than 90 days, were not related with any indications of poisonousness, and no unfavorably susceptible response have been seen when it is utilized as a sugar.

Materials and Methods
I have Purchased 15 stevia plants from CIMAP Lucknow and Cultivation and Harvesting in our home Garden, following two months I was gathered fitting measure of leaves from our Gardens. Leaf and plants Authentication by Dr. Narendra Kumar Scientist CIMAP Lucknow UP India

HPTLC Finger Print Profile of ethanolic extract of *Stevia rebaudiana*
Application-Linomat 5 Application (camag)
Volume applied : 10 µl
Solvent System-Ethylacetate: methanol: water (7.5:1.5:1.0)
Scan Wavelength-254 nm, 366 nm
TLC Plate Development-Pre-saturated Camag Twin Through Chamber

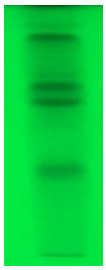

Fig 3: TLC plate at 254 nm

Fig 4: TLC plate at 366 nm

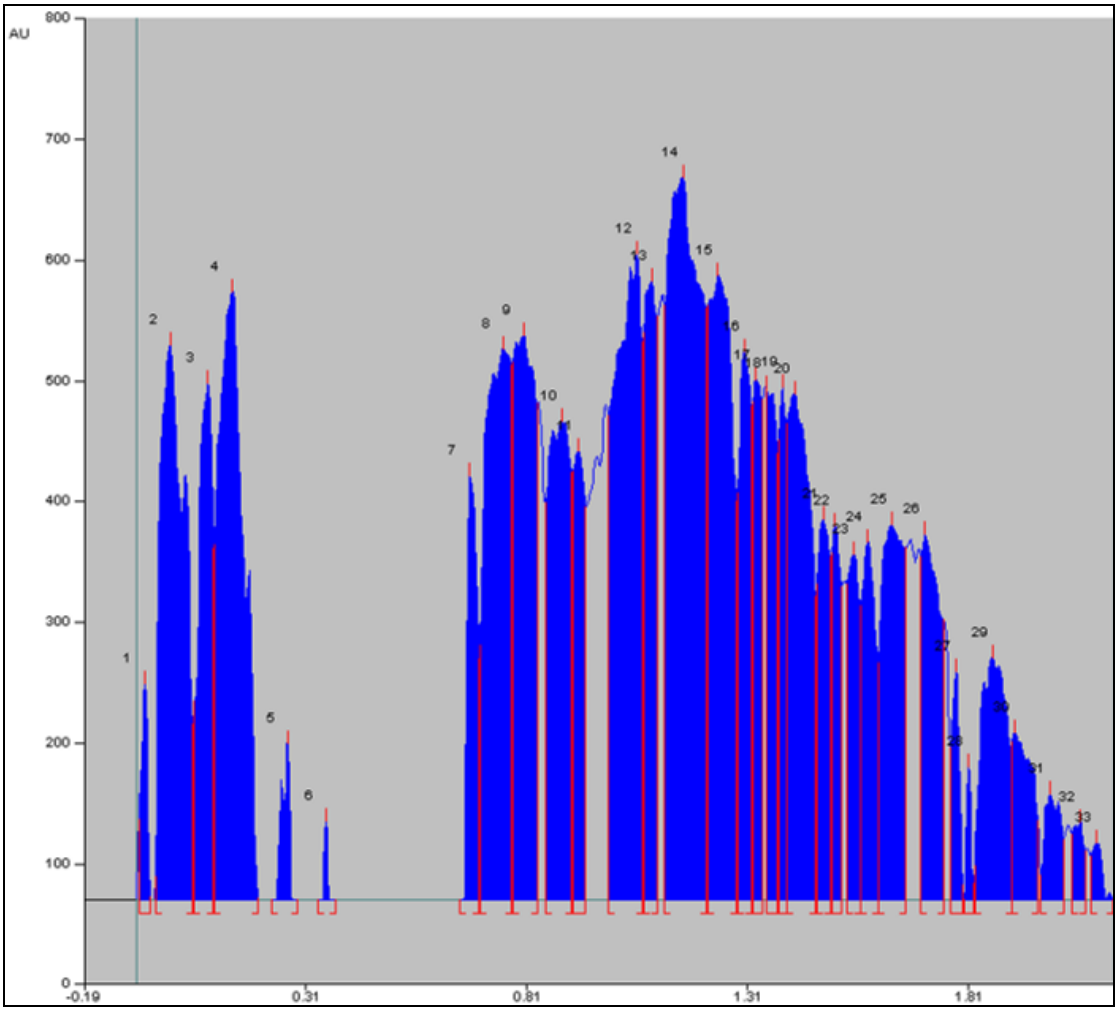

Fig 5: Peak analysis at 254 nm

Table 1: Peak Details at 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	66.3 AU	-0.05 Rf	179.4 AU	1.66 %	-0.04 Rf	2.4 AU	1269.3 AU	0.62 %	unknown *
2	-0.03 Rf	18.8 AU	0.00 Rf	459.5 AU	4.26 %	0.05 Rf	45.7 AU	12078.5 AU	5.86 %	unknown *
3	0.06 Rf	164.9 AU	0.09 Rf	428.1 AU	3.97 %	0.10 Rf	30.4 AU	6311.1 AU	3.06 %	unknown *
4	0.10 Rf	294.5 AU	0.15 Rf	503.7 AU	4.67 %	0.20 Rf	1.5 AU	14091.6 AU	6.83 %	unknown *
5	0.24 Rf	0.0 AU	0.27 Rf	130.1 AU	1.21 %	0.29 Rf	0.0 AU	1033.7 AU	0.50 %	unknown *
6	0.34 Rf	0.0 AU	0.36 Rf	65.0 AU	0.60 %	0.38 Rf	0.0 AU	225.3 AU	0.11 %	unknown *
7	0.66 Rf	0.0 AU	0.68 Rf	351.8 AU	3.26 %	0.70 Rf	39.4 AU	3819.0 AU	1.85 %	unknown *
8	0.71 Rf	211.0 AU	0.76 Rf	456.6 AU	4.23 %	0.78 Rf	42.4 AU	12835.1 AU	6.22 %	unknown *
9	0.78 Rf	445.6 AU	0.80 Rf	467.2 AU	4.33 %	0.84 Rf	11.0 AU	10747.6 AU	5.21 %	unknown *
10	0.85 Rf	328.8 AU	0.89 Rf	397.0 AU	3.68 %	0.91 Rf	53.3 AU	9793.9 AU	4.75 %	unknown *
11	0.92 Rf	354.4 AU	0.93 Rf	371.3 AU	3.44 %	0.95 Rf	25.4 AU	4637.8 AU	2.25 %	unknown *
12	1.00 Rf	401.3 AU	1.06 Rf	535.5 AU	4.97 %	1.07 Rf	31.8 AU	15576.6 AU	7.55 %	unknown *
13	1.08 Rf	469.5 AU	1.10 Rf	512.3 AU	4.75 %	1.11 Rf	33.7 AU	6959.7 AU	3.37 %	unknown *
14	1.12 Rf	492.2 AU	1.17 Rf	598.4 AU	5.55 %	1.22 Rf	30.3 AU	22260.1 AU	10.79 %	unknown *
15	1.22 Rf	491.9 AU	1.25 Rf	517.3 AU	4.80 %	1.29 Rf	30.3 AU	13572.3 AU	6.58 %	unknown *
16	1.29 Rf	337.2 AU	1.30 Rf	453.8 AU	4.21 %	1.32 Rf	38.8 AU	5866.3 AU	2.84 %	unknown *
17	1.32 Rf	412.5 AU	1.33 Rf	430.8 AU	3.99 %	1.35 Rf	14.7 AU	4226.8 AU	2.05 %	unknown *
18	1.35 Rf	423.5 AU	1.35 Rf	423.5 AU	3.93 %	1.38 Rf	39.3 AU	4885.3 AU	2.37 %	unknown *
19	1.38 Rf	380.6 AU	1.39 Rf	424.6 AU	3.94 %	1.40 Rf	32.7 AU	3235.9 AU	1.57 %	unknown *
20	1.40 Rf	394.8 AU	1.42 Rf	419.5 AU	3.89 %	1.47 Rf	51.0 AU	10328.9 AU	5.01 %	unknown *
21	1.47 Rf	261.7 AU	1.48 Rf	315.0 AU	2.92 %	1.50 Rf	34.3 AU	4170.8 AU	2.02 %	unknown *
22	1.50 Rf	287.6 AU	1.51 Rf	309.7 AU	2.87 %	1.53 Rf	58.9 AU	3171.1 AU	1.54 %	unknown *
23	1.54 Rf	261.5 AU	1.55 Rf	286.1 AU	2.65 %	1.57 Rf	39.7 AU	3771.5 AU	1.83 %	unknown *
24	1.57 Rf	243.4 AU	1.58 Rf	296.4 AU	2.75 %	1.61 Rf	33.0 AU	4372.2 AU	2.12 %	unknown *
25	1.61 Rf	195.8 AU	1.64 Rf	310.8 AU	2.88 %	1.67 Rf	31.5 AU	7529.7 AU	3.65 %	unknown *
26	1.70 Rf	284.4 AU	1.71 Rf	303.5 AU	2.81 %	1.75 Rf	32.2 AU	5968.4 AU	2.89 %	unknown *
27	1.77 Rf	126.3 AU	1.78 Rf	189.5 AU	1.76 %	1.80 Rf	12.2 AU	1579.9 AU	0.77 %	unknown *
28	1.80 Rf	0.0 AU	1.81 Rf	109.9 AU	1.02 %	1.82 Rf	13.9 AU	481.0 AU	0.23 %	unknown *
29	1.83 Rf	28.7 AU	1.87 Rf	201.1 AU	1.86 %	1.91 Rf	26.5 AU	5685.7 AU	2.76 %	unknown *
30	1.91 Rf	131.0 AU	1.92 Rf	138.2 AU	1.28 %	1.97 Rf	35.7 AU	2910.3 AU	1.41 %	unknown *
31	1.98 Rf	20.0 AU	2.00 Rf	87.7 AU	0.81 %	2.03 Rf	49.5 AU	1547.9 AU	0.75 %	unknown *
32	2.05 Rf	54.5 AU	2.06 Rf	64.5 AU	0.60 %	2.08 Rf	40.7 AU	789.4 AU	0.38 %	unknown *
33	2.09 Rf	36.4 AU	2.10 Rf	47.0 AU	0.44 %	2.14 Rf	0.5 AU	544.6 AU	0.26 %	unknown *

Pharmacognostic identification teste, microscopic Characters study are perform a very carefully in Pharmaceutical Chemistry Lab. at BMS College of Pharmacy Nasratpur Tiloi, Distt. Amethi 229309 Uttar Pradesh India. And HPTLC study are perform a very carefully in NBRI, LUCKNOW

Assortment
Aerial pieces of stevia leaf. are gathered in and around Lucknow CIMAP.

Starter phytochemical tests
The subjective phytochemical tests were completed for phenols, flavonoids, steroids, triterpenes, diterpenes, lactones, tannins, lignins, saponins, alkaloids following the techniques for Gibbs (1974) [31] 10, Kleipool (1952) [32] 11, Peach and Tracey (1959) [33] 31.

Tender loving care studies
TLC fingerprinting profile conveyed according to (Stahl E 1965) 32.

Physicochemical and fluorescence studies
Organoleptic Characters: The current examination contains concentrates on both physical and tangible attributes like tone, sensation, taste, sleek stain, and adhesive of the species under investigation. Assurance of Total Ash: Two grams of powdered medication were burned in a sintered silica pot by continuously expanding heat up to 450 ºC until the medication is liberated from carbon and afterward cooled. This debris kept in a desiccator for 15-20 min. what's more, gauged utilizing Renamed Electronic equilibrium, India and noted down the readings 14. % debris = z-x × 100/y Where, weight of void cauldron = x, weight of plant material = y, weight of pot + debris = z, weight of debris = z-x.

Assurance of acid insoluble ash
Total debris acquired was bubbled for 15 min. in 25 ml of hydrochloric corrosive and separated to gather the insoluble matter on Whatman channel paper and touched off in a sintered pot.
It was permitted to cool and afterward kept in a desiccator for 15 min.
The buildup was said something Renamed Electronic equilibrium, and the corrosive dissolvable debris was determined utilizing the recipe. % corrosive insoluble debris = z-x × 100/y Where, weight of void pot = x load of plant material = y, weight of cauldron + debris = z, weight of debris = z-x.

Assurance of extractive values
Hundred gram of powdered plant material of the two plants under investigation were removed with ethanol (95%) and water utilizing cold extraction. Subsequently got extricates were permitted to dry to room temperature. After complete vanishing, weight, nature, and shade of the concentrates were recorded 33. % Extractive worth = Weight of the buildup got × 100/Weight of plant material taken

Fluorescence Studies
The fluorescent investigation of the powder of Steviarebaudiana leaves was treated with synthetics like Ethanol, Methanol, Toluene, HNO3, concentrated H2SO4, concentrated HCl, and Water. The powdered materials gave distinctive shading responses with various synthetics, and fluorescent shades of treated and untreated medications were seen under apparent and UV light, and the perceptions were noted 34.

Histological studies
For the motivation behind contemplating the microscopical characters, freehand segments were utilized. These segments were washed in faucet water and stained with saffranine for additional perceptions, and Powder microscopy was likewise noticed and shot utilizing Magnus magnifying instrument 17.
Powder Microscopy Studies: The powdered plant material was absorbed 10% Nitric corrosive short-term. The example is washed with refined water the next day. Slides are set up by staining the doused plant material with saffranine and saw under a magnifying instrument, and the pictures were caught. RESULTS AND DISCUSSION: Preliminary Phytochemical Tests: The outcomes demonstrated the presence of the multitude of 6 metabolites tried by showing a positive reaction to the tests led

Arrangement of leaf material
Leaves of the chosen plant were culled and washed completely with running faucet water. It was again washed with sterile refined water to eliminate soil before drying measure. The leaves were dried in conceal at room temperature for seven days to eliminate the dampness content and powdered utilizing a blender processor. At last, the powdered example was put away at room temperature for additional examinations.

Fig 6: Dry Leaves of Stevia Rebaudiana

Planning of plant separate
2.5 g of powdered example was taken in water/airproof containers. To this, 50 ml of various solvents like ethanol, methanol, CH3)2CO and refined water was added. Following 2 days, the substance was mixed well completely and sifted utilizing Whatman No.1 channel paper. The filtrate was gathered and put away in a sterile container at 4°C until additional utilization. For antibacterial investigations, each concentrate was set up by dissolving

250 mg in 5 ml of 10% (v/v) watery dimethyl sulphoxide (DMSO).

Phytochemical examination

Newly pre-arranged leaf extricates were exposed to standard phytochemical examinations utilizing the standard procedures.10 In request to discover the presence of different phytoconstituents like alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinones, saponins, saps, glycosides, and phenols.

Arrangement of leaf material

New Leaves of Stevia were gathered and washed altogether with running faucet water. It was again washed with sterile refined water to eliminate earth before drying measure. Thereafter the leaves were dried in conceal at room temperature for seven days to eliminate the dampness content and powdered utilizing blender processor. At last, powdered example was put away at room temperature for additional examinations. Crisp drying powdered from Stevia leaves were introduced extricates (ethanol, methanol, CH3)2CO and watery) contains the presence of alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, gums, glycosides and phenols. Phytochemical examination uncovered that O. tenuiflorum contained rich wellspring of bioactive mixtures like alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinone, saponins, tars, glycosides and phenols. Phytochemicals are partitioned into two gatherings, which are essential and optional constituents; as indicated by their capacities in plant digestion. Essential constituents include basic sugars, amino acids, proteins and chlorophyll while optional constituents comprise of alkaloids, terpenoids and phenolic compounds.34 O. tenuiflorum leaf removes contained different phytochemical mixtures like saponins, alkaloids, flavonoids, heart glycosides, steroids, phenols and tannins.13 The presence of tannins proposes the capacity of this plant to assume a significant part as anti diarrhoea and antihaemorrhagic agent.35 The presence of glycosides has been utilized for more than two centuries as energizers in instances of cardiovascular failure.36

Preparation of plant extract

2.5 g of powdered sample was taken in air tight bottles. To this, 50 ml of different solvents such as ethanol, methanol, acetone and distilled water was added. After 2 days, the contents were stirred well thoroughly and filtered using Whatmann No.1 filter paper. The filtrate was collected and stored in sterile bottle at 4°C until further use. For antibacterial studies, each extract was prepared by dissolving 250 mg in 5 ml of 10% (v/v) aqueous dimethyl sulphoxide (DMSO).

Phytochemical analysis

Freshly prepared leaf extracts were subjected to standard phytochemical analyses using standard procedure [10]. In order to find out the presence of various phytoconstituents such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols.

Preparation of Leaf Material

Fresh Leaves of *Stevia* were collected and washed thoroughly with running tap water. It was again washed with sterile distilled water to remove dirt prior to drying process. Afterwards the leaves were dried in shade at room temperature for a week to remove the moisture content and powdered using mixer grinder. Finally, powdered sample was stored at room temperature for further studies. Fresh drying powdered from *Stevia* leaves were presented extracts (ethanol, methanol, acetone and aqueous) contains the presence of alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols.

Phytochemical analysis revealed that *O. tenuiflorum* contained rich source of bioactive compounds such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenoids and phenolic compounds [34]. *O. tenuiflorum* leaf extracts contained various phytochemical compounds such as saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins [13]. The presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent [35]. The presence of glycosides has been used for over two centuries as stimulants in cases of cardiac failure [36].

Fig 7: Stevia Rebaudiana

TLC Studies: In the current examination when the example was seen under apparent light, showed two groups. At the point when seen under short/long UV light, subsequent to showering with 10% Ethanolic H2SO4 it showed 5 groups separately. When showered with anisaldehyde 8 groups were seen.

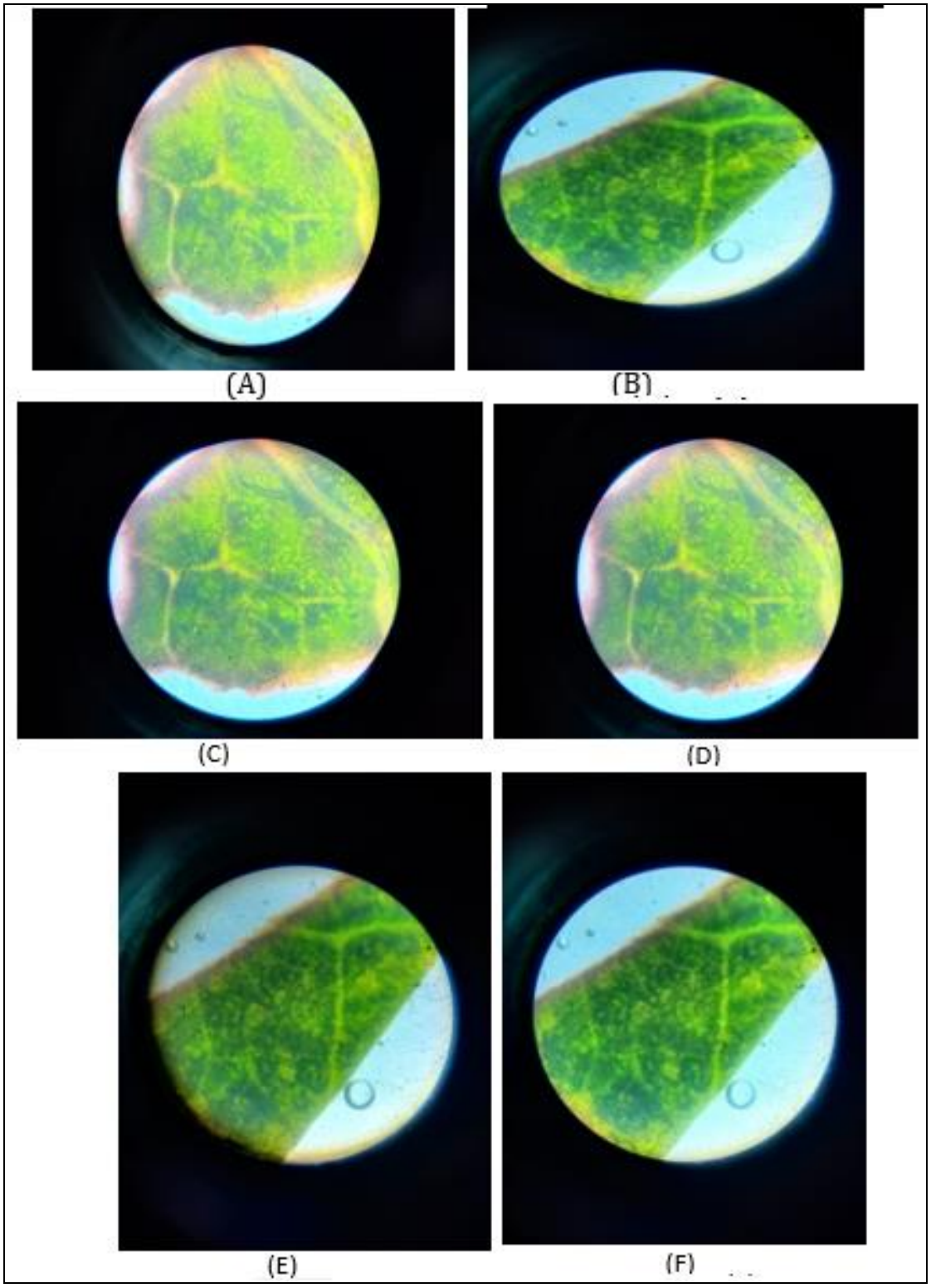

Fig 8: Microscopic Characterization of Stevia Leaves

Table 2

Morphological Study of Stevia Rebaudiana Leaves	
Colour	Green, White
Taste	Sweet, Slightly Bitter, After taste,
Smell	Pleasant,
Odour	Fragrant
Other	The epidermis of a leaf edge is covered with multicellular hairs and organs. The cells of the upper epidermis are with flimsy, twisted dividers, few stomata, anisocytosis type.

Test for alkaloids

Mayer's test-5 mg concentrate of Mithi (Tulsi) was moved in the test cylinder and afterward added 1% hydrochloric corrosive HCl, the acquired arrangement was delicately warmed. Red tone demonstrates the presence of alkaloids since Potassium mercuric iodine is available in Mayer's reagent.

Wagner's test

In this test 5 mg concentrate of stevia was taken in a test tube then 0.5 of Wagner reagent was included an answer shaken well. The appearance of ruddy earthy colored shading showing that alkaloids are available. Ruddy earthy colored tone because of iodine frames a complex is insoluble and has the shading earthy colored reddish.

Dragendorff test

5 mg concentrate of Stevia was taken in a tube. And afterward, one drop of dragendroff reagent was added to the test tube. orange-red tone, showing the presence of alkaloids. Dragendroff reagent was arranged utilizing Bismuth nitrate, Nitric corrosive, iodine, and water due to these synthetic compounds it gives orange-red color in the presence of alkaloids.

Test for flavonoids

Shinoda test: Firstly 5mg concentrate was included in the test tube then, at that point, the modest quantity of magnesium was blended in this arrangement, additionally added a couple of drops of concentrated Hydrochloric corrosive. It should shows the pink tone with the flavonoids. Shadings fluctuating from orange to red demonstrated flavones, red to ruby showed flavonoids, dark red to red demonstrated flavanones. Catechins when treated with vanillin arrangement in hydrochloric corrosive give a red-pink tone.

Lead ethanoate test for flavonoids

put 5 mg of watery concentrate of Mithi tulsi in a test tube then 1ml of lead ethanoate arrangement was added. It gives the buff shaded arrangement if the alkaloids are available.

Sodium hydroxide test for flavonoids

5 mg remove was taken in this 1 ml of the 10% arrangement of sodium hydroxide was added for the appearance of yellow shading arrangement after option of 1ml of weakening. Hydrochloric corrosive, within the sight of alkaloids the shading ought to be changed from yellow to lackluster after option of 2 ml of weakening hydrochloric corrosive.

Soluble reagent test for flavonoids

5 mg concentrate of Stevia was put in the test tube blended than the 2ml of 2% arrangement of Sodium hydroxide was poured in it, if the development of yellow which transformed into dreary after expansion of few drops of weakened acidic.

It implies that alkaloids are available in the sacred basil.

Ferric chloride test

Ferric chloride test was performed for checking the presence of flavonoids in the fluid concentrate of Stevia Leaf. First and foremost 5 mg remove was blended in with 1ml of refined water then 0.5ml of weakening alkali arrangement was added into it. After the expansion of weakened alkali few drops of concentrated Sulfuric corrosive were blended later. Development of yellowish with flavonoids.

Test for glycoside

Liebermann's test-Liebermann's test for the investigation of a glycoside is available or not in the watery concentrate of Stevia leaf in this test 5 mg concentrate of Stevia was blended appropriately with 2ml of chloroform and afterward, 2ml of acidic corrosive were blended in the. An arrangement that it was cooled in ice. In the wake of cooling 1 ml of concentrated Sulfuric corrosive was added. The shading will be changed from violet to green with the presence of alkaloids in the concentrate.

Salkowski's test for the investigation of glycoside

2ml of chloroform was with 1ml of concentrate. Then, at that point 2ml of concentrated Sulfuric corrosive were added and shaken tenderly. A rosy earthy colored shading demonstrated the presence of glycoside.

Keller-kilani test

For heart glycosides-for the affirmation of the glycoside in the concentrate 5 mg separate was taken in the test tubes then the 1 ml of frosty acidic corrosive was added. Not many drops of 2% arrangement of ferric chloride were blended into it. Then, at that point, 1 ml of concentrated Sulfuric corrosive were into the blend. An earthy colored shading ring at the edge will be framed in the presence of cardiovascular glycosides

Test for tannins

Ferric chloride test-5 mg watery concentrate of Stevia Leaf was blended in with 0.5 ml of ferric chloride arrangement. Development of blackish hasten within the sight of tannin.

Gelatine test

Was performed for checking the presence of tannin in the concentrate.

In this test, 5 mg remove was blended in with gelatine, and 1ml of water was added into the arrangement. White encourage ought to be created.

Lead acetic acid derivation test

was performed to appraise the presence of tannin where 5 mg of test tests was taken in test tubes. Hardly any drops of fundamental lead acetic acid derivation were included in the example arrangement, if earthy colored massive accelerate will be discovered it implies tannin are available in the test.

Test for saponins

Froth test was performed for distinguishing proof of saponin in the watery concentrate in which 1ml concentrate was broken down into 5ml of refined water. After the expansion of refined water, it was shaken for legitimate blending till froth was noticed. Hardly any froth was added with 2 drops of olive oil and it was shaken enthusiastically. It ought to be created emulsion with the saponins.

Test for oil

Stain test-few amount of fluid concentrate was spread onto the channel paper development of oil on the channel paper will demonstrate the presence of oil in watery.

Saponification test

Few drops of alcoholic potassium hydroxide and 0.5 ml of concentrate were taken into a test tube and blended well. 1-4 drops of phenolphthalein were added in with the general mish-mash arrangement. It was warmed on water for shower hours for 60 minutes. Arrangement of the fractional balance of soluble base which shows the presence of oils and fats.

Test for starches

Benedict's test benedict's reagent was taken for the examination of carb. the 5 mg remove was blended in with few drops of benedict's reagent than permitted to heated up,

the ruddy earthy colored accelerate are found with the presence of the sugars .

Molisch's test
At first 5 mg remove was taken in a test tube then the 1 ml of Molisch's reagent was added into it. The blend was shaken appropriately. From that point forward, 2ml of concentrated Sulfuric corrosive was poured cautiously at the edge of the test tube. The appearance of a violet ring at the interface showed the presence of a carb.

Test for steroids
5 mg concentrate of Stevia leaf was blended in with 1 ml of chloroform then a couple of drops of concentrated Sulfuric corrosive and acidic corrosive were added into it. The greenish shading has demonstrated the presence of steroids.

Salkowski's test
3 drops of concentrated sulphuric corrosive were added into the 5 mg separate. The development of red tone shows the presence of steroids. Test for proteins-Biuret's test-5 mg separate was added with a couple of drops of biuret's reagent. The got combination was shaken well and permitted to warm for 1-5 min. The appearance of red or violet shading demonstrated the presence of proteins

Million's test
5 mg remove was blended in with 2ml of Mallon's reagent. The arrangement was warmed for 5 min red shading hastened transforms into red shading which affirmed the presence of protein

Ninhydrin test
Fluid concentrate of tulsi was blended in with 2 ml of 0.2% arrangement of Ninhydrin and bubbled for 2 min on water shower if violet tone showed up with the presence of amino acids and proteins in the watery concentrate.

Table 3

Name of the test	Reagents	Ethanol Extract	Methanol Extract	Acetone Extract	Aqueous Extract
Test for Alkaloids	Dragendorff,s Wagners Hagers	+ +	+ + +	+	+ + + +
Phenolic compounds	%Ferric chloride solution+2% potassium Ferrocyanide	-			
Test for Tannins	Ferric chloride reagent	+ + + +	+ + + +	+ +	-
Test for Flavonoids	Potassium Dichromate Gelatin	+ + + + +	+ + + +	+ + +	+ + + +
Test for steroids	Alkaline Reagent 10% Lead Acetate	+ + + +	+++++	++++	+++++
Test for cardiac Glycoside, Steviosol	Salkowki,s test	+ + + +	+ + + + + +	+	+ + + + +
	Keller –kilani test	+ + + + +	+ + + + + +	+ + + +	+ + +
Test for Saponins	Froth test	+ + + + +	+ + + +	+ + +	+ + +

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