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Phytochemical screening of aqueous extract derived from *Stevia rebaudiana* leaves, *Musa acuminata* fruits and *Solanum lycopersicum* fruits

Shom Prakash Kushwaha[1, 2*], Sujeet Kumar Gupta[3], Sushil Kumar[2]

[1] School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road, Moradabad, Uttar Pradesh, India

[2] Faculty of Pharmacy, Integral University, Dasauli, Kursi Road, Lucknow, Uttar Pradesh, India

[3] Department of Pharmaceutical Chemistry, Hygia Institute of Pharmaceutical Education and Research, Ghaila Road, Gazipur Balram Road, Lucknow, Uttar Pradesh, India

Abstract

The process for detecting secondary metabolites is known as phytochemical screening. This aids the separation and detection of chemical components for discovery of new therapeutic agents. The aqueous extract derived from stevia leaves, tomato fruits and banana fruits was subjected to phytochemical screening. The chemical tests for alkaloids, carbohydrates, glycosides, flavonoids, saponins, steroids, triterpenoids, phenolic compounds, tannins, xantho proteins, amino acids, fats, fixed oils and volatile oils revealed that the secondary metabolites like flavonoids and phenolic compounds were present significantly. Phenolic compounds act as antioxidants and known to lower the blood sugar levels by inhibiting α-amylase. Flavonoids act as antioxidant and undergo preferential oxidation to safe guard other antioxidants like ascorbic acid. The plethora of biologically active constituents obtained in the extract paves the path for its further toxicological/pharmacological screening. The developed prophylactic/therapeutic formulation may be beneficial in the management of free radical mediated disorders.

Keywords: stevia, tomato, banana, phenols, flavonoids

Introduction

Stevia rebaudiana Bertoni leaves are been employed for sucrose substitution [1] and offer similar levels of satiety as compared to higher calorie sucrose [2]. A teaspoonful of dried stevia leaves has a sweetness equal to a cup of sucrose [3]. Stevia is accompanied with benefits of zero calorie [4] intake, reduced body weight and is a potential plant for treatment or prevention of various diseases [5]. Banana fruit belonging to the family Musaceae [6] is beneficial in high blood pressure [7] and improves blood sugar control [8]. Tomato is a powerful antioxidant [9] and lowers the oxidative stress by ripping off the reactive oxygen species to reduce the oxidative damage to lipid (membrane lipid, lipoprotein), protein (enzyme) and genetic material [10]. We performed the aqueous extraction to obtain an extract derived from stevia leaves, tomato fruits and banana fruits. The phytochemical screening for alkaloids, carbohydrates, glycosides, flavonoids, saponins, steroids, triterpenoids, phenolic compounds, tannins, xantho proteins, amino acids, fats, fixed oils and volatile oils was executed.

Materials and methods

Plant Materials: *Stevia rebaudiana* Bertoni. Leaves (family: Asteraceae), *Musa acuminata* Colla. fruits (family: Musaceae) and *Solanum lycopersicum* L. fruits (family: Solanaceae) were obtained from the local market of Lucknow, India and subjected to authentication (IU/PHAR/HRB/20/03, IU/PHAR/HRB/20/01, IU/PHAR/HRB/20/02) at Faculty of Pharmacy, Integral University, Lucknow, India.

Chemicals: Molish's reagent, Fehling's reagent, Barfoed's reactant, Benedict's reagent, Ferric chloride, Mayer's reagent, Wagner's reagent, Dragendroff's reagent were purchased from S.D. Fine Chem. Ltd., India.

Results and Discussion

Plants are rich source of secondary metabolites and finds huge scope as neutraceutical and medicine. Phytochemical screening of the extract derived from stevia leaves, tomato fruits and banana fruits exhibited significant presence of phenolics and flavonoids. Saponins, tannins, carbohydrates and amino acids were present in noticeable amount while glycosides, alkaloids and volatile oils were slightly present. Steroids, triterpenoids, fats and fixed oils were absent as conveyed by the chemical tests. Flavonoids are naturally occurring phenolic compounds with well-established beneficial properties to human health. Flavonoids are antioxidant, build up capillary walls, diminishes osteoporosis, recovers blood cholesterol and drops risk of coronary heart diseases. Phenolic compounds have a high affinity to chelate metals, guard cells and body chemicals against free radicals [11]. Phenolic compounds are reported to deactivate the growth of tumors. Water soluble compounds demonstrate a plethora of beneficial biological activities. Catechin increases low density lipoprotein resistance to oxidation [12], quercetin promotes overall cardiovascular health [13]. Phenolic compounds lower blood sugar levels by inhibiting α-amylase [14]. Flavonoids prevent the oxidation of antioxidants like ascorbic acid by undergoing privileged oxidation [15].

The screened extract may be beneficial for maintaining health and in free radical mediated disorders. It may prove safe enough from side effects as observed in synthetic medicines [16].

Table 1: Chemical tests for the aqueous extract derived from stevia leaves, tomato fruits and banana fruits. Presence of secondary metabolite assigned as +++ (Significantly present), ++ (Present), + (Slightly present), - (Absent).

Class	Test	Experimental	Observation	Inference
Alkaloids (50 mg of extract dissolved in dilute HCl and filtered)	Mayer's Test	2 drops of Mayer's reagent and 0.2 mL of extract aqueous solution added through the ends of the test tubes	Yellowish white precipitate	+
	Wagner's test	0.2 mL of extract aqueous solution + Wagner's reagent	Reddish brown precipitate	+
	Dragendroff's test	0.2 mL of extract aqueous solution + Dragendroff's reagent	Orange red precipitate	+
	Hager's test	0.2 mL of extract aqueous solution + Hager's reagent	Yellow colour precipitate	+
Carbohydrates	Molish's Test	0.2 mL of extract aqueous solution + 2 drops alpha napthol solution + 0.2 mL of concentrated sulphuric acid slowly along the sides of the test tubes, cooled in ice water and left to stand	Violet ring at the point where two fluids intersects	++ Carbohydrates
	Fehling's test	Boiled 0.2 mL of extract aqueous solution with 0.2 mL of each Fehling's solution A and B in a water bath	Red precipitate	++ Reducing sugars
	Barfoed's reactant	0.2 mL Barfoed's reactant added to 0.2 mL of extract aqueous solution and placed in a boiling water bath for 2 minutes	Brick red precipitate	++ Reducing sugars
Glycosides	Legal's test	0.2 mL of the extract dissolved in pyridine. Sodium nitroprusside solution added and made alkaline using 10% sodium hydroxide solution	Pink colour	+
	Baljet's test	0.2 mL of the extract aqueous solution + 0.2 mL sodium picrate solution	Yellow colour	+ aglycon moiety
	Keller - kiliani's test	0.2 mL of alcoholic extract of drug + equal volume of water + 0.2 mL of strong lead acetate solution → shake → filter → filtrate extracted with same amount of $CHCl_3$ → $CHCl_3$ extract evaporated to dry → remainder dissolved in 0.5 mL of glacial acetic acid + few drops of $FeCl_3$ solution + 0.5 mL of conc. H_2SO_4	Reddish brown layer turns to bluish green	+ aglycon moiety
Flavonoids	Base-acid test	0.2 mL extract aqueous solution + drops of concentrated sodium hydroxide + drops of dilute HCl	Yellow colour (NaOH) and then colourless (HCl)	+++
	Shinoda's test	0.2 g of extract + 1 ribbon of Mg and dilute HCl + waited for 3 minutes. After cooling, added 0.5 mL of absolute alcohol	Pink colour	+++
Saponins	Foam test	0.2 mL of extract aqueous solution was shaken vigorously	Froth	++
Steroids and triterpenoids	Salkowoski's test	0.1 g of dried extract + dissolved in chloroform in a dry test tube + 0.5 mL conc. sulphuric acid from sides of test tube	Absence of brown / yellow colour in the lower layer	-
Phenolic compounds	Ferric chloride test	0.2 mL of extract aqueous solution + few drops of alcoholic 5% ferric chloride solution	Blue-black colour	+++
Tannins	Lead acetate test	0.2 mL of extract aqueous solution + 0.02 g lead acetate and shaken well	White turbidity	++
Xantho proteins	Nitric acid test	0.2 mL of the extract aqueous solution + 0.2 mL of conc nitric acid	Yellow colour	++
Amino acids	Ninhydrin test	0.2 mL of the extract aqueous solution + 0.2 mL of ninhydrin solution	Pink color	++
Fats and fixed oils	Potassium bisulfite test	Heated 0.2 mL of extract aqueous solution + few crystals of potassium bisulfite	Absence of pungent odour	-
Volatile oils	Sudan III test	0.2 mL of the extract aqueous solution + 0.2 mL of Sudan III solution	Pink colour	+

Conclusions

Herbal dosage types are herbal forms (liquid, solid, semi-solid) in a specific formula with or without excipients (such as decoctions, tablets and ointments). The plethora of biologically active constituents in the studied extract paves the path for further toxicological/pharmacological screening and formulation. The formulation may be aimed at the development of a prophylactic/therapeutic option for the management of free radical mediated disorders such as diabetes.

Conflict of interest

The authors declare no conflict of interest.

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