Herbal Tea Formulation for Health Rejenuvation: Nutritional, Physicochemical and Sensory Analysis

S J Kabilan, R Baskar, G Poorani

Abstract*:*The aim of the study were to perform nutritional, phytochemical and sensory analysis and assessing the potential of herbal formulation made up of Wedeliachinensis leaves and Boerhaaviadiffusa roots in possessing phytochemical contents and to be a part of herbal tea development. The phytochemical analysis were performed for each herb sample to determine the total phenolics, total flavanoids, crude alkaloids, tannins and identification of phytoconstituents such as phytosterol, coumarin, terpenoids, anthraquinone and phlobatannins. As part of nutritional analysis, tests such as moisture content, ash content and mineral composition of the herbal formulation mixture were performed. GC/MS screening was performed to check the presence of several effective compounds which indicated that selected sample holds the potential to be stated under "Nutraceuticals". Formulation of herbal tea with the selected herbs was made and sensory analysis performed to validate the aroma, colour, astringency, flavor and overall acceptability as an herbal tea. The results of the nutritional, phytochemical showed that the formulation mixture of herbs Wedeliachinensis and Boerhaaviadiffusa roots is an excellent source of nutraceuticals with high therapeutical importance. Multiple beneficial phytochemical content featured in this polyherbal formulation, makes it as a perfect physical and psychological health rejuvenator.

Keywords*: *Boerhaaviadiffusa, Polyherbal formulation, tisanes, Wedeliachinensis.

I. INTRODUCTION

Herbal teas are normally mixtures of multiple ingredients, and are more precisely known as 'tisanes'. Tisanes are combinations of dried leaves, grasses, seeds, nuts, fruits, barks, flowers, or other botanical elements which gives them their unique taste and the potentials of health benefits. Most of the herbal teas usually consist of one main herbal ingredient or a blend of herbal ingredients, promised to provide a specific purpose, such as rejuvenation or relief from a specific condition [16].

Wedeliachinensis (Manjalkarisalankanni in Tamil), Asteraceae is a highly regarded herbal medicine in Siddha, Unani and Ayurvedic system of medicine [5]. Extensive studies shows presence of flavanoids, diterpenes, triterpenes saponins and phytosteroids [6]. It is also reported to possess antioxidant, analgesic, anti-inflammatory, antimicrobial, CNS depressant,hepatoprotective, wound healing, antistress and anticancer activity [8,18].

Boerhaaviadiffusa (Mookirattaikeerai in Tamil) is one among the well known medicinal plants that are used to treat huge multiple human diseased conditions as mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita [11]. Huge variety of phytochemicals like flavonoids, alkaloids, glycosides, rotenoids, steroids, lipids, triterpenoids, lignans, proteins, carbohydrates and glycoproteins etc have been reported from the herb [12]. The promising therapeutic effects of this plant include diuretic, hepatoprotective, anti-inflammatory, anti-cancer, anti-fibrinolytic, immuno-modulatory, anti-diabetic, immuno-suppressive, analgesic, anti-lymphoproliferative and used for the treatment of TB [15,17,22].

The aim of this study is to develop a herbal tea formulation that possess high nutritive value and also to evaluate this herbal formulation by performing nutritional, sensory and phytochemical analysis.

II. MATERIALS AND METHODS

A. Chemicals

methanol, ethanol, ferric chloride, TPTZ, acetate buffer, hydrochloric acid, quercetin, sodium nitrite, $AlCl_3$, sodium hydroxide, FC reagent, sodium carbonate, catechol, tannic acid, formic acid, theophylline, acetic acid, SPI solution.

B. Sample collection and Extraction

Sample collection:

Wedeliachinensis leaves and *Boerhaaviadiffusa* root powder were collected from an FSSAI approved herbal powder manufacturer from Coimbatore, Tamil Nadu. All herbs were stored in sealed container for further use. The samples were labeled as the *Wedeliachinensis* leaves (WC), *Boerhaaviadiffusa* (BD) and Tea formulation mix (TF MIX).

Sample extraction:

About 20g of powdered TF MIX of these herbs was extracted with 150 ml of distilled water. Then it is allowed to evaporate in open air to obtain aqueous extracts.

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C. Nutritional analysis

Determination of Moisture content

The moisture content of the TF MIX formulation was measured based on AACC method. Two gramsof formulation powder was placed over a weighed petri plate and dried using hot air oven at 120°C for 2 hours. After drying the petri plate weight was taken and the contents were cooled and then petri plate was re-weighed. The drop in the weight was calculated as percentage of moisture content.

Moisture content (%) = [(W1- W2)/ W1] × 100

W1 = Weight (g) of Sample before drying.

W2 = Weight (g) of Sample after drying.

Determination of Ash content

The ash content of the TF MIX formulation was quantified, explained by AACC method. Twograms of theformulation powder was placed in a pre weighed crucible and then the incineration process undertakes in a muffle furnace at 420 °C for 5 hours and then crucible was cooled and then weighed.

Ash (%) = [Weight of ash / Weight of Sample] × 100

Determination of mineral composition

Inductively coupled plasma Mass spectrometry (ICP-MS), is used to analyze the composition of minerals in the sample.

D. Phytochemical analysis

Estimation of flavanoids (Shirazhi et al., 2014)

Samples were dissolved in their respective solvents (1mg/ml) to serve as stock. 25 µl of the formulation mix was taken and made up to 2.5 ml with distilled water. To the formulation mix 75 µl of 5% sodium nitrite solution was added and incubated at room temperature for 5 minutes. After incubation 150 µl of 10% $AlCl_3$ was added and incubated at room temperature for 6 minutes. 0.5 ml of 1M sodium hydroxide was added and the colored complex formed was read at 510 nm. Quercetin standard was used to prepare a calibration curve. The results were denoted as mg quercetin equivalents / g extract.

Estimation of Total phenol content (Siddiqui et al., 2017)

Sample was prepared by dissolving the extracts in their respective solvents (1mg/ml). 0.1ml of the sample was taken and made up to 2ml with distilled water. 0.5 ml of FC reagent was added and the tubes were incubated for 3 minutes. After the incubation period, 2ml of sodium carbonate was added and the tubes were kept in water bath for one minute at 50ºC. Then the absorbance of the colored complex formed was read at 650 nm. Catechol was used as standard to draw the calibration curve. The results were denoted as catechol equivalents / g extract.

Estimation of Tannins

0.2ml of the samples dissolved in their corresponding solvents were taken (1mg/ml). 0.25ml of sodium carbonate solution was added to all the samples and 0.2ml of distilled was taken as blank. All the tubes were made up to 5ml with distilled water and incubated at room temperature for 90 minutes. The absorbance was read at 260 nm. Tannic acid standard was used.

Estimation of Anthocyanin

0.5ml of the sample was taken and 4ml of formic acid was added and the absorbance was read at 530 nm.

Anthocyanin content (mg/ 100 g of dry matter)
= (A x MW x DF x 1000) / (ε x 1)

Where A = Absorbance

DF = Dilution factor

MW = Molecular weight of cyaniding-3-glucoside chloride (C21H21ClO11. 449.2)

W = Weight of the sample

ε = Molar absorptivity (26.900)

Estimation of Alkaloids

1.5ml of each sample was taken in boiling tubes and 1ml of 0.01M SPI solution and 0.5 ml 0.1M acetic acid solution was added. 10 ml of distilled water was added to all the boiling tubes and was kept in the boiling water bath for 10 minutes. After incubation, 2ml of the 0.01M MBTH solution was added to all the tubes and kept in the boiling water bath for 2 minutes. The tubes were then cooled and made up to 25 ml with distilled water. The complex formed was read at 630 nm. Theophylline was used as a standard at a concentration of 1 mg/ml. It was prepared by dissolving 10 mg of theophylline in 20 ml of methanol and then it was made upto 100 ml with distilled water. The results were expressed as mg theophylline equivalents / g extract.

E. Sensory analysis

Selection of panelists

Twenty-five (25) panelists were recruited from Kumaraguru College of Technology campus for the acceptance tests. Panelists were mostly students aged between 18 and 26 years with few college staff.

About 30 ml of each herbal infusion was served in a 50 ml transparent glass cup. One sample was served at a time. Panelists were discouraged from conferring among one another during the analyses. The herbal infusions were approximately around 70 °C at the time of tasting.

The panelists were instructed to score their acceptance for 6 attributes of the infusions: colour, aroma, flavor, aftertaste, astringency and overall acceptability. The panelists scored their acceptance of the attributes using a 9-point hedonic scale with 1 meaning ‘dislike extremely’ and ‘9’ meaning ‘like extremely’.

III. Result And Discussion

Nutritional analysis

Determination of Moisture content

Moisture content affects the food products stability and storage, etc. The moisture content was calculated for TF MIX which is reported in Table I.

Table I: Moisture content estimation for TF MIX

Moisture content %	2.66 %

The moisture content value for tisanes should be less than 11% (Kirk and Sawyer, 1997). If the value is above the prescribed limit, the tea formulation will be liable to mould infestation. As the value is below the prescribed level, the shelf life of the product is unaffected by the moisture content.

Determination of Ash content

Ash content analysis is another important parameter, because it also directly influences thestability, nutritional content of the food and its storage, etc. Ash represents the inorganic residue that remainswhen the water and organic matter is removed by heating, which indicates the measure of the total amount of minerals present in a food. The ash content was calculated for TF MIX which is reported in Table II.

Table II: Ash content estimation for TF MIX

Ash content	10.95 %

The ash content value for tisanes should be between in the range of 4% to 14%.

Determination of mineral composition

The quantity of minerals present in the TF MIX is as tabulated in the Table III. An appreciable amount of sodium was present in TF MIX, which can play a key function in maintaining the loss of sodium due to diuretic action of the herbs, whereas magnesium has numerous health utilities including mediation of nerve impulses, regulation of body temperature,detoxification, production of energy, cardiac disorders and the growth of strong bones and teeth (Nordin et al., 1990). Another mineral is aluminium, which was present in high amount, helps in increasing immunity. High potassium helps in treating kidney disorders, lowering blood pressure, increasing water flow in body and mainly acting as an anti-diabetic. The other trace minerals such as chromium, manganese, iron, zinc, copper and molybdenum were present in little amounts, which will exhibit their own therapeutic potentials ranging from boosting immunity, detoxification to being anti-carcinogenic and improving brain function (Liao et al., 1998).

Table III:Estimation of Mineral content for TF MIX

S.No	**Analyte**	**Concentration (ppm)**
1	Na	4501.99
2	Mg	17210.69
3	Al	4539.98
4	K	109181.22
5	Cr	0.0014
6	Mn	0.0409
7	Fe	0.3793
8	Cu	0.0024
9	Zn	0.0041
10	Mo	0.35

The complete absence of toxic heavy metals selenium, arsenic, lead and cadmium makes this product, free of any toxicity and a safely consumable one. The presence of fair amount of mineral content makes this product a potent nutraceutical.

Phytochemical analysis

Table IV represents the contents of flavonoids, total phenol, tannins, alkaloids and anthocyanins in aqueous extract of TF MIX.

Phenolic compounds depict antioxidant and can act as scavengers of free radicals. These activities in vivo may slow down the ageing processes as well as protect the human body against against diseases such as atherosclerosis, coronary diseases and cancer (Lee et al., 1993). Flavonoids,is one of the polyphenolic compounds, that is widely present in vegetables and fruits. Multiple positive health effects have been reported such as anti-cancer, anti-inflammatory effects, anti-viral and to minimize the risk of cardiovascular diseases (Vessal et al., 2003). These activities are generally associated with antioxidant properties of flavonoids. The number of flavonoid derivatives is more than 4000 and their antioxidant properties are very different. Alkaloids are a class of nonnutritive phytochemical compounds that are synthesized as secondary metabolites by the plant cells (Cody et al., 1986). They fight against free radicals and are capable of quenching their activity. The presence of phytoconstituents like flavonoids, alkaloids and others was estimated by various phytochemical assays.

The presence of these photochemical simply attributes to the antimicrobial and antioxidant activities of the tea formulation (Blytt et al., 1988). Flavonoids, major crusaders in the prevention of cancer, also possess antiviral and anti-bacterial properties.

Alkaloids are usually synthesized as secondary metabolites in the plants and thus exhibit various health benefits. Total phenols are involved in the delaying the process of ageing. Tannins and anthocyanins also contribute to antioxidant properties of plant extracts. Anthocyanins are representative of pigments present in colored fruits and flowers. Due to high consumption of anthocyanins, it would be of great interest to find out the additional biological benefits related to these compounds (Dillard et al., 2000).

Table IV: Quantification of polyphenolic compounds in TF MIX

Phytochemical tested	Value (mean ± SD) of three replicates	Units
Total Phenols	3.85 ± 0.04	Catechol equivalents (mg CE/g sample)
Flavanoids	1.6 ± 0.033	Quercetin equivalents (mg QE/ g sample)
Tannins	35.72 ± 0.01	Tannic acid equivalents (mg TAE/ g sample)
Anthocyanins	203.33 ± 0.03	Cyanadin-3-glucoside equivalents (mg C3gE/100g sample)
Alkaloids	7.51 ± 0.012	Theophylline equivalents (mg TE/g sample)

Hexadecanoic acid	57-10-3	20.575	3.201
Estra-1,3,5(10)-trien-17-one, 2-[(trimethylsilyl)amino]-3-[(trimethylsilyl)oxy]-	77883-26-2	25.907	2.139
1,5-Dimethoxy-2,4-bis(3-Methylphthalidyl)Benzol	64042-52-0	28.57	1.567
Dimethyl 2,3-bis(1,3-dimethylindol-2-yl)fumarate	72206-09-8	28.57	1.567
1,8-Bis(3,4-dicyanophenyl)anthracene	122504-53-4	28.57	1.567
2-Butenoic acid, 2-methyl-	77573-09-2	41.881	0.116
n-Butyryl-3-demethylthiocolchicine	123643-58-3	41.881	0.116
Benzeneacetic acid, 3,4-dimethoxy-, trimethylsilyl ester	27750-60-3	42.144	0.162
4-Cyanonaphtho[1,2-b]thiophene	107971-16-4	44.343	0.996

GC-MS analysis

The secondary metabolites in the TF MIX were determined by GC-MS. Analysis of aqueous extract of TF MIX resulted in detection of phytochemicals and common fatty acid. The details of some noted compounds are tabluated in Table V. The main compounds were myo-inositol, which is the most common form of inositol, is a sugar alcohol found in nearly all plants and animals (Downes and Macphee., 1990). Myo-inositol decreases high blood pressure and sugar levels. Myoinositol has antioxidant, anti-inflammatory, and immune-enhancing properties.

Hexadecanoic acidpossesses mild antioxidant and anti-atherosclerotic properties, which also add texture and mouth, feel to processed foods (Mancini et al., 2015).

The presence of neronine, an alkaloid imparts antimicrobial and antioxidant activities. Butenoic acid has numerous beneficial effects in humans on energy homeostasis and related diseases such as diabetes and obesity and also it has pronounced antimicrobial and anticarcinogenic effects.

Benzene acetic acid helps in relaxing mood and treating brain disorders. Presence of various other compounds such as 4, 5 – cyclopenteno-1, 2-diazine, p - hydroxystyrene, cyclohexasiloxane increases the health benefits of the formulation.

Table V:TF MIX extracts GCMS profiling

Compound Name	Cas #	RT	% Area
4,5-cyclopenteno-1,2-diazine	6250-96-0	6.334	2.199
N-Benzyl-2-phenethylamine	3647-71-0	6.334	2.199
p-hydroxystyrene	2628-17-3	6.334	2.199
Cyclohexasiloxane, dodecamethyl	540-97-6	11.475	1.01
3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	72439-84-0	13.731	0.86
Myo-Inositol, 4-C-methyl-	472-95-7	14.658	84.834
Neronine	1167-58-4	16.482	1.935

Sensory analysis

Sensory evaluation of the herbal infusion was performed for overall acceptance (colour, aroma, Flavor, astringency and after taste) by 20 members on the basis of 9-point Hedonic scale.

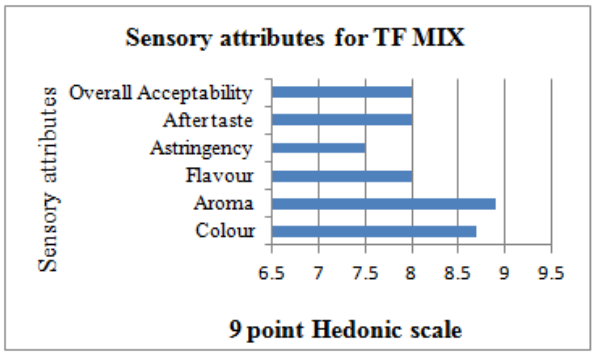

Figure 1: Sensorial attributes of formulated herbal tea

From the above sensory data the average of overall acceptability was around 8 which means 'like very much' as per the 9 point hedonic scale. Thus, the sensory attributes makes the herbal tea eligible for a commercial consumable with high scores from evaluators for its colour and aroma. The astringency created by it was least liked by the evaluators.

IV. CONCLUSION

The results of the nutritional, phytochemical profile proved that the formulation mixture of herbs *Wedeliachinensis* and *Boerhaaviadiffusa* roots is an excellent source of nutraceuticals with high therapeutical importance. Enormous health benefits

highlighted in the polyherbal formulation shape it as anideal physical and mental health rejuvenator. The low economy community can benefit best out of this, to improve their health. Hence, the above herbal infusion can be used as a better alternative to flavored teas which can render health gains too.

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Recent Publications:

- **Baskar,R.,** Akshaya, S.B., Akshitha, R., Dhilip Kumar, G. , Poorani, G. (2018).Evaluation of antioxidant and phytochemical activity in solvent extracts of *Delonix regia*.International Journal of Green Pharmacy, Volume 12(3), July-September issue, S607-S616.
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Notable Publications:

INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES (2019) IF 4.78 A novel metalloprotease from banana peel and its biochemical characterization

MATERIAL SCIENCE AND ENGINEERING C (2019) IF 5.08 Biological synergy of greener gold nanoparticles by using Coleus aromaticus leaf extract

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JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B: BIOLOGY (2018) IF 4.06 Improved Conductivity and Antibacterial activity of poly (2- aminothiophenol) - silver nanocomposite against human pathogens

JOURNAL OF MICROENCAPSULATION (2016) IF 2.04 Formulation, characterization, in vitro and in vivo evaluation of castor oil based self-nano emulsifying levosulpiride delivery systems