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Sudeshna Shyam Choudhury
Assistant Professor and Head, Department of Microbiology, St. Xavier's College, Kolkata, West Bengal, India

Jaydip Ghosh
Assistant Professor and Head, Department of Microbiology, St. Xavier's College, Kolkata, India

Arun Kumar Jana
Principal Engineer, C-DAC, PLOT E2/1, Block GP, Sector V, Salt Lake, Kolkata, West Bengal, India

Sejuti Roy
Junior Research Fellow, Microbiology Department, St. Xavier's College, Kolkata, West Bengal, India

Renu Kumari Prasad
Post Graduate Student, Microbiology Department, St. Xavier's College, Kolkata, West Bengal, India

Anushree Pratihar
Post Graduate Student, MAKAUT University, Kolkata, West Bengal, India

Corresponding Author:
Sudeshna Shyam Choudhury
Assistant Professor and Head, Department of Microbiology, St. Xavier's College, Kolkata, West Bengal, India

Development of therapeutic index of different types of herbal and medicinal teas

Sudeshna Shyam Choudhury, Jaydip Ghosh, Arun Kumar Jana, Sejuti Roy, Renu Kumari Prasad and Anushree Pratihar

Abstract
Normal tea Camellia sinensis extract is renowned for its therapeutic potential in terms of antioxidant, antimicrobial and anticancer values. The volatile and nonvolatile secondary metabolites like catechins, tannins are responsible for such properties. Herbal teas (tisanes) are herbal infusions are beverages made from the infusion or decoction of herbs, spices, or other plant material in hot water. Chamomile, lavender, lemongrass, jasmine, hibiscus, butterfly blue pea extracts of all the medicinally valid plant extracts (either shoot or from flower) are carrying therapeutically important active secondary metabolites to show antioxidant, antimicrobial, anticancer values. The infusions made from them have enormous commercial values. Once all those infusions are mixed with normal tea it increases the therapeutic values of the former and called as medicinal tea. The evaluation of therapeutic values of all the commercially available medicinal and herbal teas could be done with antioxidant, antimicrobial and anticancer value assay methods. Then further the total responsible candidates like non-volatile and volatile active secondary metabolites could be scored with E-sensor based detection techniques like E-nose and E-tongue. The correlation analysis of antioxidant, antimicrobial and anticancer data with E-nose and E-tongue score helps to derive the therapeutic indices of Medicinal and Herbal teas. Moreover thus it would help to validate the therapeutic potentials of unknown samples of medicinal and herbal teas based upon the indices derived from E-nose (volatile) and E-tongue (nonvolatile/soluble) data.

Keywords: Medicinal tea, herbal tea, antioxidant, antimicrobial, anticancer, e-nose, e-tongue, therapeutic index

Introduction
Tea (*Camellia sinensis*) has enormous medicinal/therapeutic value. Many reports are there which supports its antioxidants, antimicrobial (antibacterial, antiviral, antiparasitic), anticancer values of tea which makes it as a medicinal beverage. The active secondary metabolites like flavonoids and its derivatives like Catechins (specially in Green and White tea-ECGC-epigallocatechin gallate), tannins (in Oolong and Black tea-Theaflavins and thearubigin) of different types of tea are responsible candidate for its medicinal properties. Black, Green, Oolong and White tea differs according to their fermentation processes so their active secondary metabolites are also variable. Sharangi, 2009 [5] elaborately had discussed about anticancer (lung cancer. colon cancer, stomach cancer, urinary bladder cancer) properties of tea polyphenols.

Medicinally important secondary metabolites are also available from other different phyto/herbal resources. Medicinal tea are the beverages where along with normal tea base (*Camellia sinensis*) such herbal infusions are mixed to increase the medicinal potential of the beverages.

Herbal teas are herbal infusions are beverages made from the infusion or decoction of herbs, spices, or other plant material in hot water. The term "herbal tea" is often used in contrast to true teas e.g., black, green, white, oolong which are prepared from the cured leaves of the tea plant, *Camellia sinensis* by different fermentation processes. They are called tisanes. A tisane is an infusion of fragrant and medicinally relevant herbs, fruit, bark, flowers, or spices that is steeped or simmered in hot water. The health benefits of herbal teas were discussed by Ravikumar, 2014 [1]. The antioxidants and vitamins found in herbal teas are great for helping fight disease and infections. They can protect against oxidative stress and lower the risk of chronic disease and shows antioxidant anti-aging properties, reduces blood pressure and inflammation.

Some herbal teas are Chamomile tea, Hibiscus tea, Rooibos tea, lemongrass tea. Dried Chamomile flowers from the plant *Matricaria chamomilla* are used to make Chamomile tea which has antidiabetic and anticancer activity, Therapeutic effects of Chamomile extracts were described by Miraj and Alesaeidi, 2016 [6]. The *Aspalathus linearis* leaves are used to make herbal Rooibos tea that is mainly originated in South Africa and shows antioxidant activity. Medicinal effects of Roibos tea was discussed by Canda *et al.*, 2014 [7]. Hibiscus tea is made from the Hibiscus flower can show antipyretic action and act against sore throat. Riaz and Chopra, 2018 [8] had discussed about phytochemistry and therapeutic uses of hibiscus extracts. Ginger tea is a herbal beverage that is made from ginger root, it is traditionally used as ethnomedicine. Butterfly pea flower tea, commonly known as blue tea, is a caffeine-free herbal tea, or tisane, beverage made from a decoction or infusion of the flower petals or even whole flower of the *Clitoria ternatea* plant. Lakshan *et al.*, 2019 [9] had demonstrated the commercial potential of the butterfly pea extracts considering its therapeutic potential as beverage. *L. angustifolia* and *L. latifolia*. are two types of flower are components of Lavandin hybrid plant of Lavender/Lavendula commonly used shows medicinal properties reported by Koulivand *et al.*, 2013 [11]. Therapeutic uses of lemongrass (*Cymbopogon citratus*) extracts were described by Shah *et al.*, 2011 [12].

Individually all those herbal extracts can be mixed with normal tea to make Chamomile green or white tea, Hibiscus green tea like "medicinal tea". Tulsi green tea is such a medicinal tea which can boost up immune system and acts as antioxidant by incorporating Tulsi leaves (*Ocimum tenuiflorum*). Jamsime tea has benefits against neurodegenerative disorders like Alzhimer's disease. Once the Jasmine flower extract (*Jasminum officinale*) is mixed with normal tea the medicinal value is increased. Medicinal effects of Tulsi Green tea and Jasmine green tea were described by Chakraborty *et al.*, 2014 [10].

The volatile and nonvolatile secondary metabolites are responsible to show the therapeutic potentials in all herbal and medicinal teas. The bioinstrumentation techniques like LC-MS, GC-MS could thus help to identify and characterize the nonvolatile (soluble) and volatile components. But in all those cases the crude extracts needs to be purified which is the task of drug designing. But there are so many E-sensors like E-nose and E-tongue which collectively give a score against total non-volatile and volatile active components of medicinally important plant extracts and bevarages.

Our aim of work is to find out the antimicrobial (by measuring zone of inhibition in solid agar medium against different microbes), antioxidant (by DPPH reduction assay), anticancer (by measuring cytotoxicity analysis on SHY5Y cell line) properties of all those medicinal and herbal teas and score the E-sensor based values with E-nose and E -tongue, then with regression analysis, by correlation of the antimicrobial and antioxidant data, the therapeutic indices could be established. Moreover to compare with the raw medicinal plant part extracts with commercially available processed herbal teas. The therapeutic indices generated from the E-sensors could thus help to commercially and therapeutically validate commercially available medicinally important beverages.

Materials and Methods

Materials taken

Tea: Normal Green, Black, White and Oolong tea were taken.

Medicinal Tea: Three medicinal tea like Lavender Chamomile White tea, Jasmine Hibiscus green tea, Tulsi Green tea.

Fig A: Different Medicinal teas

Herbal Tea: five herbal teas or tisanes like Chamomile tea, Lavender tea, Rooibos tea, Hibiscus tea, Blue Pea Chamomile tea were analysed to develop therapeutic index. Tulsi lemongrass and Tulsi ginger tea is used to validate the therapeutic indices derived from Enose E tongue data with their corresponding antioxidant and antimicrobial potential.

Fig B: Different herbal teas

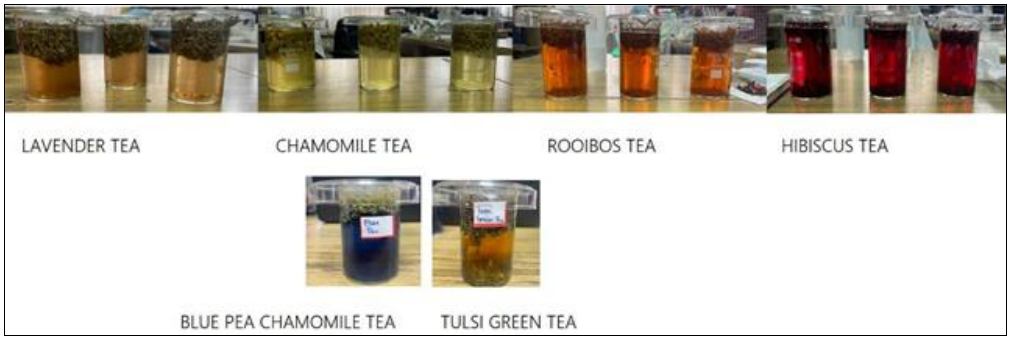

Fig C: Aqous extracts of different herbal and medicinal teas

Preparation of extracts

All the samples were extracted in water, Ethanol and Methanol.

Antioxidant assay

The antioxidant assay were done according to Rebeiro, *et al.*, 2002 [3] by taking 0.4% of DPPH solution in methanol and water. The bleaching of violet colour of DPPH was recorded at 517nm and the % DPPH reduction/mg fresh wt. gives % antioxidant potential. The standard used was Vitamin C tablets - (Fig 1, Fig 5)

Antimicrobial assay

The antimicrobial testes were done according to Bose and Bose, 2008 [2] by doing Agar cup plate method against *E. coli*. *Bacillus subtilis*, *Streptococcus* sp. and the zone of inhibition were measured after 24 hours. Ampicillin solution (5mg/ml) is taken as positive control and as negative control solvents in each case - (Fig 2a, 2b Fig 5)

Anticancer assay

The anticancer studies were done with MTT assay in SHY5Y cell line - (Fig 6)

E-nose and E tongue data analysis

The E-nose data were determined in terms of Taster's score and Noraroma index, E-tongue data were determined in terms of taster index. (Table 1 and 2, Fig 5)

Regression analysis

With antioxidant, antimicrobial and anticancer values individually as dependant variable and Nor aroma indices (determined from E nose machine) independent variables therapeutic indices were evaluated, same thigs were applicable to E tongue data that is T.S indices (Fig 3, Fig 4)

Results and Discussion

Antioxidant potential is also highest for Lavender Chamomile white tea 96.08±.1%, which is higher than white tea 94.0±.1%, (Fig 1)

Jasmine Hibiscus green tea is showing highest antimicrobial activities (zone of inhibition against *Streptococcus* sp. is 0.9±0.01 cm higher than normal green tea that is 0.8±0.01 cm) against *E. coli.* Lavender chamomile white tea which is 0.85±0.01 cm which is higher than normal white tea which is 0.69±0.01 cm (Fig 2).

Anticancer assay showed IC_{50} value for oolong tea is 100µg/ml (Fig 6), Chamomile Lavender White tea is showing higher cell protection activity.

Noraroma index determined by E-nose showed higher score for Lavender tea and Jasmine Hibiscus Green tea (19.96 for Lavender tea, 17.7 for Jasmine Hibiscus Green tea)-Table 1.

E tongue data shows Taster's score is highest for Lavender tea 9.12. (Table 2)

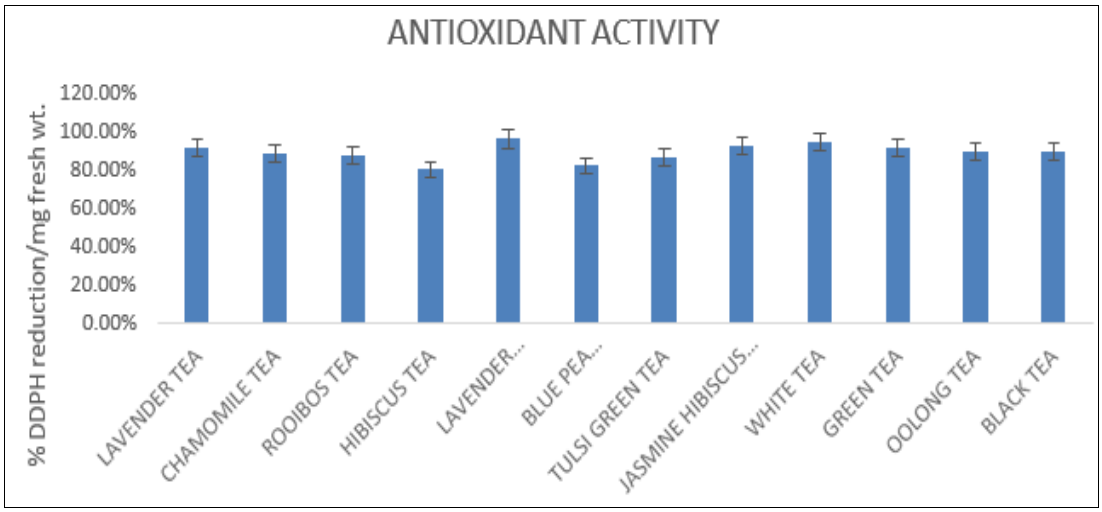

Fig 1: Antioxidant activities of different medicinal and herbal teas (aqueous extracts)

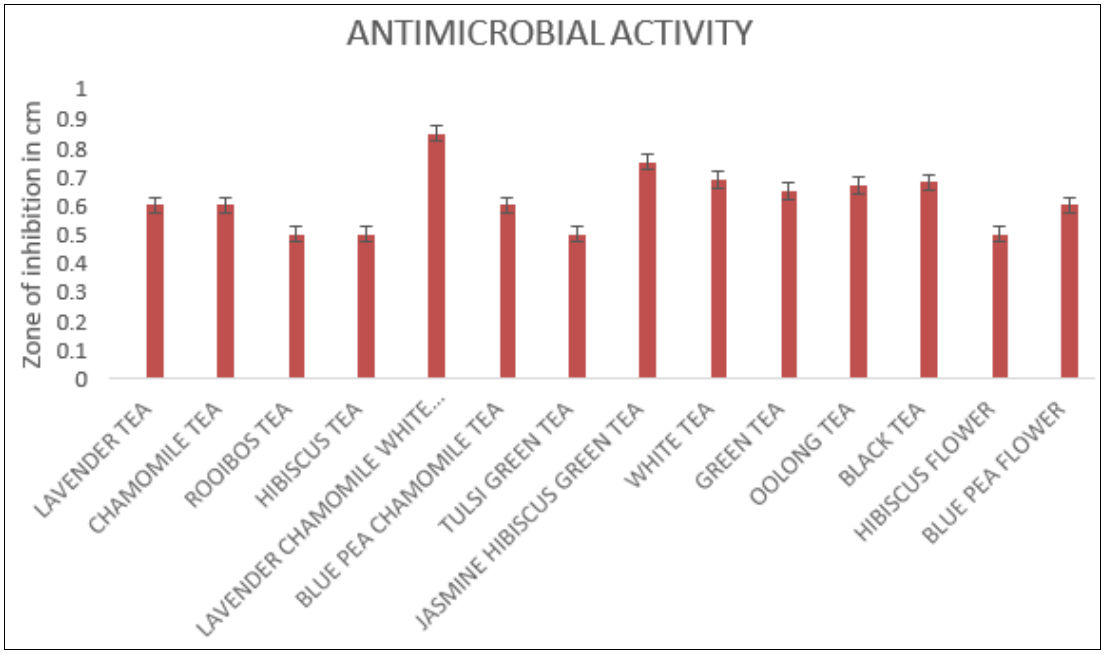

Fig 2a: Antimicrobial activities of different medicinal and herbal teas (aqueous extracts) against *E. coli.*

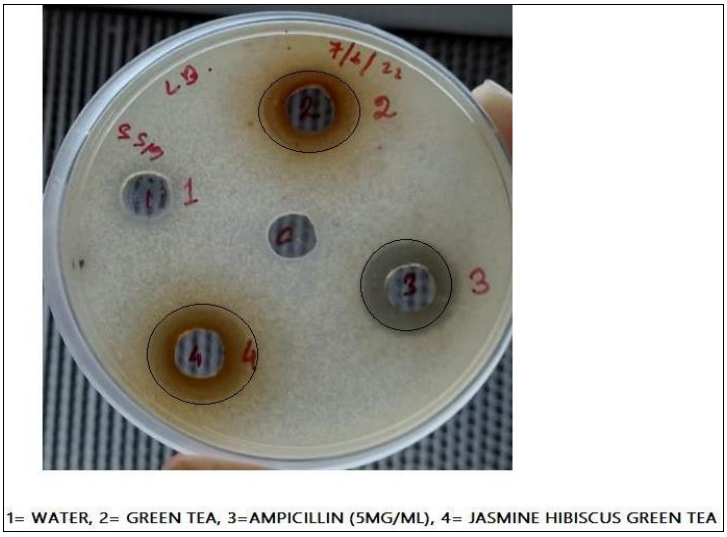

Fig 2a: Antimicrobial activities of green tea and Jasmine hibiscus green tea (aqueous extracts) against *E. coli.*

Moreover the raw plant extracts (flower extracts of Hibiscus and Blue pea chamomile) show higher antioxidant and antimicrobial activities than processed commercially available Tisanes. Further with Regression analysis the therapeutic score could be developed by correlating antioxidant, antimicrobial, anticancer data with E-nose and E-tongue scores.

Table 1: E nose data

Tea Types	T.I
Lavender tea	19.96
Chamomile tea	10.66
Rooibos tea	12.68
Hibiscus tea	17.13
Lavender chamomile white tea	5.46
Blue pea chamomile tea	6.63
Tulsi green tea	5.7
Jasmine hibiscus green tea	17.7
White tea	16.5
Green tea	14.3
Oolong tea	13.9
Black tea	12

N.I= Nor aroma Index

Table 2: E-tongue data

Tea Types	T.I
Lavender Tea	1.93
Chamomile Tea	2
Rooibos Tea	2.16
Hibiscus Tea Lavender Chamomile	2.2
White Tea	1.46
Blue Pea Chamomile Tea	1.73
Tulsi Green Tea Jasmine Hibiscus	1.6
Green Tea	1.76
White Tea	1.39
Green Tea	1.29
Oolong Tea	1.25
Black Tea	1.19

T.I=Taster's Index

Regression analysis reveals that with known data the following result is available.

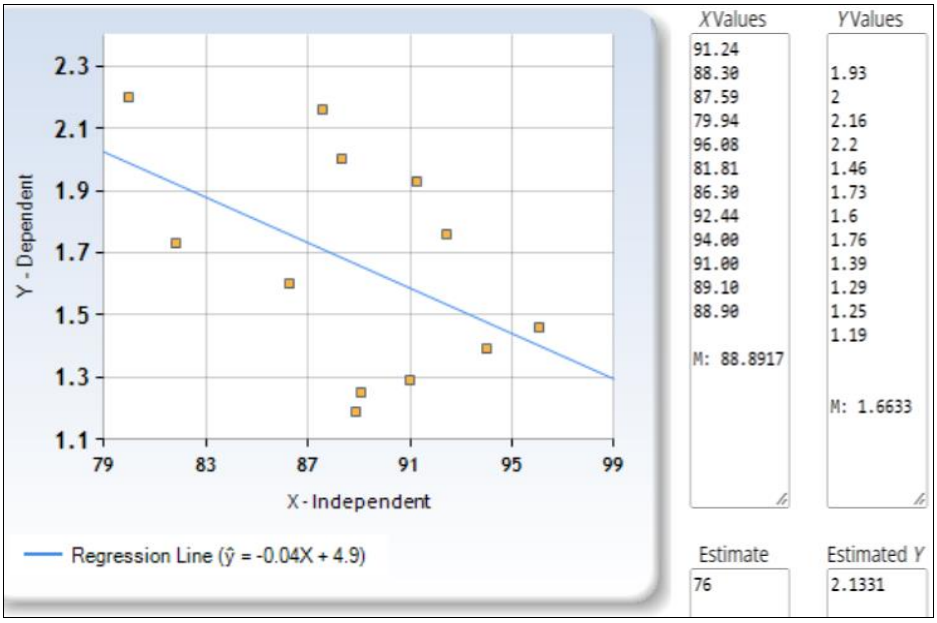

Fig 3: Regression analysis to determine tentative Taster's index of Tulsi lemongrass tea based on experimental antioxidant potential

Now antioxidant potential of Tulsi lemongrass tea was determined with experiment is 76%, from regression analysis the presumed Taster's index would be 2.21 (Fig 3), practically it was observed 2.2, so this could be established as therapeutic index, S.D is 0.004

Similarly the E tongue data was first derived for Tulsi ginger tea, that was 2.1 which corresponds to 86.14% antioxidant activity, which is further experimentally validated (86%), S.D is 0.07 (Fig 4)

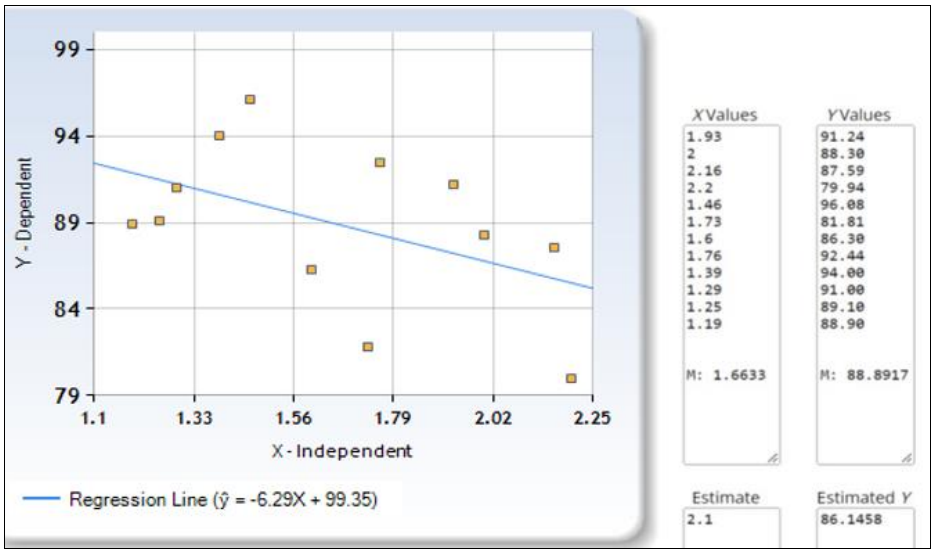

Fig 4: Regression analysis to determine tentative antioxidant potential of Tusli ginger tea based on independent variable experimentally derived E-tongue data

Similar type of data was used by Veloso *et al.*, 2018 [4] to evaluate the quality of honey. Similarly E nose data is also used to validate and derive therapeutic index of different types of medicinal and herbal teas with other therapeutic properties like antimicrobial and anticancer activities.

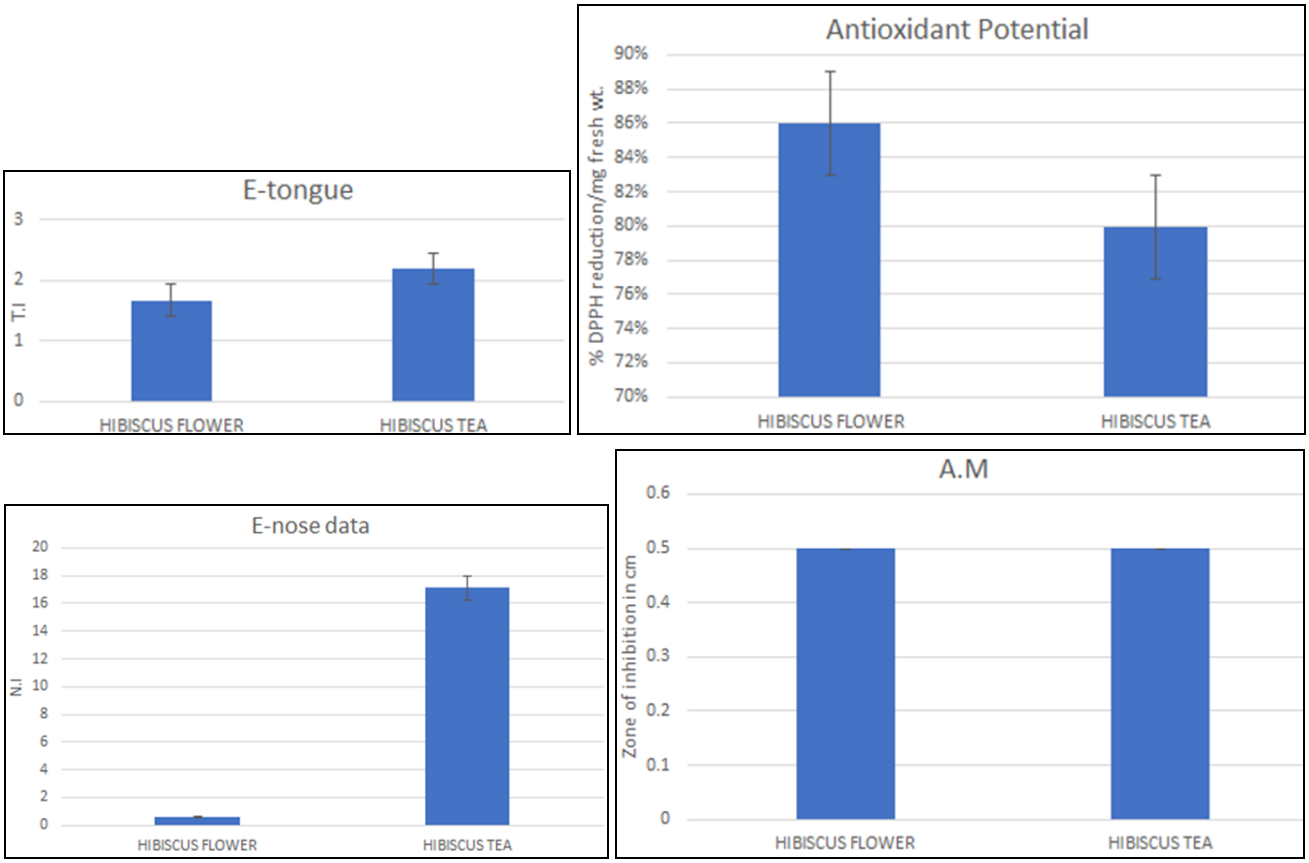

Fig 5: Comparison of Antioxidant potential, antimicrobial potential, E-nose and E-tongue data of Hibiscus tea and Hibiscus flower

E-tongue value (T.I) is higher in hibiscus tea than flower; Antioxidant potential; A.O is higher in flower than tea, Antimicrobial activities A.M are quite equivalent but E-nose value (N.I) is much more higher in values in herbal hibiscus tea than flower (Fig 5)

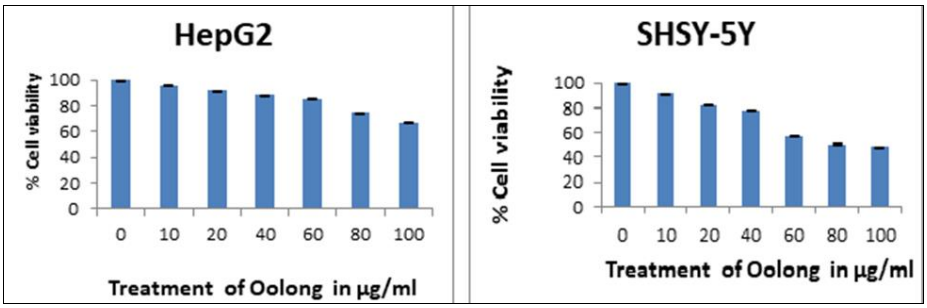

Fig 6: Anticancer assay (cytotoxicity analysis by % cell viability) against hepatic cell line normal Hep G2 and cancer cell line SHSY-5Y

The anticancer activities of oolong tea was found as IC_{50}-HepG2= 100ug/ml; SHSY-5Y=60 ug/ml according to % cell viability, so cancerous cells are more inhibited by Oolong tea, the E-tongue data of Oolong tea was found as 1.25, so 60% reduction in IC_{50} (half maximal inhibitory concentration) corresponds to Taster's index 1.25-Fig 6.

Volatile and nonvolatile components are higher and same in Hibiscus and Lavender tea. Hibiscus tea contains-protocatechuic acid (volatile) and quercetin (non-volatile)-antioxidant and antimicrobial. Lavender tea contains-linalool (volatile) & eucalyptol (nonvolatile)-antioxidant and antimicrobial. In Rooibos, chamomile tea nonvolatile components are higher. Rooibos tea contains-aspalathin, aspalalinin which are nonvolatile in nature, and Chamomile tea contains-chamazulene which is nonvolatile in nature-they are antioxidants & antimicrobial. White, Green, Oolong, Black tea has more volatile components-linalool, geraniol-Saijo and Kuwabara, 2014 [13].

The findings show that the Antimicrobial, antioxidant, E-nose and E-tongue data reveal that once green tea is mixed with hibiscus and jasmine flower extracts or infusions all those properties are increased as the antioxidant potential of Green tea is 91%; but Jasmine hibiscus green tea antioxidant potential is 92.44±0.01%, antimicrobial potential against *E. coli.* for only green tea is 0.69 but for the later one is 0.79±0.01 (Fig 2b), E-nose designating volatile components responsible to show therapeutic potential for only green tea is 14.3 N.I, but for the Jasmine hibiscus green tea it is 17.7, and for the E-tongue value for only green tea T.I is 1.29, but for the later it is 1.76, it corroborates the fact that once normal tea is fortified with herbal extracts it becomes more and more therapeutically relevant. Chakraborty *et al.*, 2014 [10]. Also described such things in terms of normal green tea and Jasmine green and Tulsi green tea.

Conclusion

The correlation analysis of antioxidant, antimicrobial and anticancer data with E-nose and E- tongue score helps to derive the therapeutic indices of Medicinal and Herbal teas. Moreover thus it would help to validate the therapeutic potentials of unknown samples of medicinal and herbal teas based upon the indices derived from E-nose (volatile) and E-tongue (nonvolatile/soluble) data. The data help collectively to find volatile and non-volatile active component nature of the herbal/medicinal tea responsible for contribution towards antioxidant and antimicrobial properties. From the known data of Flower E. tongue/E-nose data-the antioxidant and antimicrobial data of another pair could be presumed, so after processing as a form of herbal/medicinal teas the changes in biochemical components could be presumed from therapeutic index. The increase in therapeutic potential in terms of antioxidant, antimicrobial, E-nose and E-tongue data from the normal tea to herbal tea could validate the relevance of mixing of medicinally important plant extracts, infusions with the normal tea to make herbal tea. The therapeutics indices generated and validated through regression analysis could thus help to further validate medicinally important commercially available bevarages.

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