RESEARCH PAPER | Biochemistry | Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X



Antioxidants and Phytochemical Profile of Various Extracts Frombuccholzia Coriacea

KEYWORDS: Fermentedaqueous extract; Buchholziacoriacea; Phytochemicals; Methanolic extract; Antioxidants in health.

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ABSTRACT *Hospitals globally are facing unprecedented crisis due to increasingly high cost of medicinedue to limited raw materials and isolation tchniques. This study was conducted to evaluate the qualitative and quantitative anti-oxidant and phytochemical profile of Buchholziacoriacea. Distillates at varying degrees, methanolic and fermented aqueous extracts were assayed.Up to 0.003 mg/ 100glevels of hyperoside (a flavone found with potent antioxidant activity, with the potential to protect thehuman lung fibroblastfromhydrogen peroxide -induced oxidative damage), was obtained for the fermented extracts –this fell below the detection limit of the analytical instruments in other treatments. Higher values of oxalate in fermented sample may have been influenced by the profile of La caserasoda used in the fermentation. Comparatively, various formulations revealed total phytochemicals of about 1437.44 mg /100g for fermented aqueous extract, 1972.51 mg/100g for methanolic extracts, 0.006 mg/100g for la casera soda, and 0.05 mg/100g, 0.04 mg/100g and 356.17 mg/100g for the distillates of aqueous extract at 100°C, 200°C , and 300°C respectively. From the results obtained, fermentation and distillation of aqueous extracts of B.coriacea could be better means of isolating important phytochemicals in preference tomethanolic extraction; whereas methanolic extraction gave a better quantitative isolation of phytochemicals therein and the amazing composition calls for further screening.*

INTRODUCTION

Consistent consumption of vegetables, fruits, herbs and spices, over the years has been connected with lots of health benefits, though with an unclear mechanism till recent years. It has been observed that plants contain many bioactive compounds thereby giving them some medicinal/ therapeutic properties (Nwaichi et al., 2012; Himal et al., 2008). Most of these properties are embedded in their phytochemical constituents.The significance of phytochemicals in the prevention of chronic diseases like cardiovascular disease,cancer, and diabetics has been reported by Aruoma(2003). It occursinplants,giving them color, flavor, smell also constituting part of its defense system (disease resistance) naturally (Ibrahim and Fagbohun, 2012). Bibithaet al.(2002) repoted traditional use of some plants as primary health remedies owing to their pharmacological propertiesand one of such is wonderful kola (gotu kola).

Wonderful kola or musk tree,also called *Buccholziacoriacea*,is often used as herb and has acclaimed high natural medicinal endowments.

*Buchholziacoriacea*belongs to the family of *capparacea*ethe seeds (with a peppery taste)are often eaten topromote memory retention.The bark when converted into a pulp can be inhaled thereby helping in the relieve of headache, nasal congestion and sinusitisin Ivory Coast and is also used in the treatment of smallpox or skin itching in Gabon (Anowi et al., 2012). The pulped bark is applied to the chest to treat chest pains and also boils. In Liberia, skin eruption are being treated using the seeds, it also has antihelminthic properties (Anowi et al., 2012). Studies have revealed its antifungi and antibacterial effects (Mbata et al., 2009), Antispasmodic and antidiarrhoea properties (Anowi et al., 2012) and anti-Diabetic Properties (Ezeigbo, 2011). To further understand local prescription formulations from Wonderful kola, this study seeks to evaluate the levels of some active ingredients/phytochemicals in a fermented and methanolic extracts of this kola as regular local formulations for herbal therapy in Nigeria and to makecomparisons among these different local formulations of *B.coriacea*.

MATERIALS AND METHODS

SAMPLE SOURCING AND PREPARATION: Fresh wonderful kola fruits wereprocured from Mile 1 market Port Harcourt Rivers state Nigeria and air-dried after due identification. An aqueous extract was prepared and was fermented for three days before the phytochemical analysis alongside the methanolic extraction.

Fermentation of Aqueous solution of *Buchholziacoriacea*: About400g of dried *B.coriacea* was weighed, chopped into smaller sizes and re-weighed to confirm no weight loss due to fallen pieces.The chopped sample was homogenized by pounding in a mortar with pestle and was introduced into an electric blender (Sonik Japan 2012), then into 250 ml distilled water.That poured into a 1.5 litre plastic jar containing50cl soda water (la casera Soda) and was made up to mark usingdistilled water.The entire mixture was allowed to stand for three (3) days under room temperature to give way for fermentation.

Methanolic extraction of *B.coriacea*: About400g of dried *B.coriacea* was weighed, chopped into smaller sizes and re-weighed to confirm no weight loss due to fallen pieces. The chopped sample was homogenized by pounding in a mortar with pestle to increase the surface area to methanol volume ratio and was introduced into an electric blender, then into 250 ml of conc. Methanol.The mixture was introduced into a big glassware and another 250ml methanol was added and stirred properly and allowed to stand for 4 hours to allow the active ingredients to dissolve into the methanol. This was filtered into a pre - weighed flat bottom flask. The filtrate was weighed and clamped with a retort stand which was then mounted on the heating man-

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tle set at 100°C.The sample was heated until the entire methanol in the mixture got evaporated leaving the extract from *B.coriacea* which was introduced into a sterilized container under aseptic conditions and was stored in a refrigerator until it was taken for phytochemical analysis.

PHYTOCHEMICAL ANALYSIS: The phytochemicals tested for includedlignin,tannins,phytate, oxalate, phenolic acid, flavonoids,terpenes, and phytosterol in the fermented aqueous extract, methanolic extract and *la Casera* Soda water.

LIGNIN: Lignin content was determined following modified method of Liyama and Wallis (1988). An aliquot of 2ml acetyl bromide in glacial acetic acid containing perchloric acid was introduced into the brown vial containing 15mg extract. The digest was transferred to a 50ml volumetric flask containing 5ml of 2M sodium hydroxide and 12ml acetic acid concentration calculated using absorbance value and optical density following spectrophotometry.

TANNINS: Tannins content was extracted following modified method of Haslam (1966). The extracts were quantified after centrifugation in acetone and water (7:3) mixture, using the HP 6890Gas Chromatogramwith ChemStation Rev. A0901 1206 software.

PHYTATE: Phytate content was determined following modified method of Chitravadivu et al. (2009). Defatted with hexane - redistilled 0.5g filtered sample was extracted with 25ml of 3% trichloro acetic acid and 3ml of 1% $FeCl_3.6H_2O$ added before 10min centrifugation. 3ml of 1.5N NaOH and 7ml of distilled water were added to 0.1N HCl washed residue to precipitate ferric phytate. This was placed for 15min in a boiling water bath, cooled, then centrifuged. The supernatant was used for the phytate value determination.

OXALATE: Solid residuefrom 0.20 mm screenedrepeatedly crushed until the sample was free of fluid.The combined fluid was poured into the 1 litre glass jar for the calcium oxalate crystals. The crystallization continued until the supernatant was light coloured. The supernatant was removed by draining off and the formed sediment was transferred to smaller 100ml test tube for proper concentration and the solid matter was dried at 60° C.

PHENOLIC ACID: For the effective removal of the phenolic compounds, 2 stages were followed:

Stage 1:A50mg of the sample was extracted for 16 h with 5ml of NaOH(1M)at room temperature as following the method of Kelley et al. (1994) andProvan et al. (1994).The sample wastwicecentrifuged at 5000xg and the supernatantsdecanted into a glass test tube.Heatingfor 2 hours at 90°C was in place to let out the conjugated phenolic compounds.Centrifugation of titrand (4M HCl; pH < 2.0) from cool extract was done to remove the precipitate, while keeping the supernatant.

Stage 2: Extraction of theresidue from stage 1 was done using 5 mlNaOH (4M) and heated to 160°C. Cool mixture was filtered, and the residue washed with deionised water. pH (< 2.0) adjustment of supernant was done using with 4M HCl.

Purification stage: A15 ml ofcombined supernatant was passed through a pre-conditioned (by passage of 2 mlEthyl acetate then, 2ml water, pH < 2.0)elution solid - phase extraction tube,attached to a visiprepat about 5mL min^{-1}. Under 60 kPa vacuum,the tubes were placed until the resin was confirmed dried. Then the extracted Phytic acid were eluted with 1 mlEthyl acetate into GCautosampler vials. Analar grade phenolic acid standards were of Aldrich Chemicals.

FLAVONOID: This was done according to the methods of Millogo-Kone, et al. (2009). A 100ml of distilled water was added to dried extracts weighed to constant weight, and boiled for 10 mins. Also, 100ml of boiling methanol: water (70:30) v:v was adeed, allowed to macerate (4hr) and filtered. The filtrate was concentrated to 5ml for gas chromatography analysis.

TERPENES: The extraction was carried out following the modified method of Ortan et al. (2009). Samples were pulverized and terpene constituents extracted with redistilled chloroform. The terpenes were removed with 10ml of the solvent for 15 min and the mixture was filtered into vial and injection (1 µl)into the port of the GC was madefrom concentrated(1 ml) vial sample.

PHYTOSTEROL: This was carried out following the modified AOAC 994.10 and AOAC 970.51 official methods (AOAC, 2005). An aliquot of extracted oil in screw-capped test tubes. was saponified at 95°C for 30 min, using 3 ml of pre- Benzene (0.20ml) mixed KOH (10%)in ethanol. 3: 2 deionised water: hexane was employed for theextractionof the non-saponifiable materials like sterol. Using 2ml hexane, $1^1/_2$h three – times extractions were done to exhauast adequately completesterol extraction and injection (1 µl) into the port of the GC was madefrom hexane - concentrated(1 ml) vial sample.

RESULTS AND DISCUSSION

Phytochemical screening of fermented aqueous and methanolic extracts of *B. Coreacea* revealed levels of free radicals scavenger, lignin which natural anti-oxidant properties advances its use in cosmetics and topical formulations (Reviews, 2013). Good values of 16.94mg/ 100g and 18.36mg/ 100g were obtained for fermented and methanolic extracts as compared to those observed in Soyabeans (Bellaloui, 2012). Although lignin is indigestible by animal enzymes, gut bacteria are able to secrete lignasesthat can biodegrade the polymer.Up to 5.58 mg/ 100g tannins was obtained with methanolic extraction (table 2). Phytate is the main storage form of phosporus in many plant tissues.It gave comparable values of 0.019mg/ 100g and 0.021mg/ 100gfor fermented and methanolic extracts respectively. AGRIS(2013) reported phytate reduction by lactic acid fermentation of seeds, nuts and grains. Hurrell (2003) reported the less intestine - absorbable insoluble precipitates of co - binding of Fe and Zn to Phytic acid and the implications in developing countries where people rely on such mineral diets;it can therefore be considered an antinutrient, inspite ofpracticable therapeutic effects. The catalytic activity of phytase,from internal digestive microflora, on phytate, releasing metal ions or other and ehancement and intialisation of intestinal absorption however, has been reported by Famularo et al. (2005).

Oxalates however, showed higher levels in fermented extracts (0.012mg/ 100g) than in methanolic extracts (0.007mg/ 100g). This may have been influenced by la casera soda added during fermentation process. Also, results obtained showed that phenolic acid (table 5)gave the highest valueof Vnillic Acid (259.93161mg/100g)boosting further the anti-oxidant nature of fermented aqueous *Buccholziacoriacea*,giving it antiaging,hepatoproctective (Itoh

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et al., 2009), and antidiabeticproperties.The mean levels of flavonoidsin *B.coriacea*are as shown in table 6. Kaemferol, Quercetin, Isorhamnetin, Myricetin, Quercetin-3,7,4'-trimethyl ether and Isoquercetingave significant valuesand with a total flavonoids content of 327.08278mg/100g, showing a good anti-oxidative properties.Flavonoids (both flavonols and flavanols) exhibit biological activities, including antiallergenic, antiviral (Cushine and Lamb, 2005), anticancer, (antibacterial (Cushine and Lamb, 2005, 2011), antiinflammatory (Yamamoto and Gaynor, 2001), and vasodilating actions but are most commonly known for their antioxidant activity due to their reported (Pietta, 2000)abilty at free radical formation reduction and scavenging of free radicals. Marked levels of Catechin (flavan –3- ol), Kaemferol and Quercetin may suggest advantage as anticancer (lung) remedy as suchactivity has been reported by Irwin (2008). Reduced associated risk of coronary heart disease by three major actions: improving coronary vasodilation, reducing bood – clotting tendencies of platelets and shielding low density lipoproteins (LDLs) from oxidation are well documented.

Terpeneas (table 7)gave a high level of Lupeol which is known to have antiprotozoal,antimicrobial, anti-inflammatory antitumor,chemopreventive properties (Margarethand Galo,2009) and anticancer agent (Prasad et al., 2008). Generally extractsgave a total terpene content of 114.55852mg/100g and 132.41083mg/ 100g for fermented and methanolic extracts respectively.Such level of terpenes may have confered on Wonderful kola the property of an expectorant and humectant in the traditional medicine and its use in the treatment of acute or chronic bronchitis and related conditions.Cholesterol lowering potential of phytosterolshave been attributed tocompetition with cholesterol at absorption site in the gut. Weingartner(2008) however reported non – existence of evidence to any useful effect on cardiovascular disease (CVD) or overall mortality.

CONCLUSION

This study revealedgood levels of useful phytochemical constituents of *Buchholziacoriacea*which may be the rationale for diverse applications in trado-medine for therapeutic purposes. Since eleveted levels may pose toxicity symptoms, fermentation has shown reducing abilities for most phytochemicals and a good alterantive for specific ailments based on medical details. Methanolic extraction gave higher levels of these phytochemical and may be used mostly for research and not prescription.

ACKNOWLEDGEMENTS

The authors are grateful to Becky and Maureen for their assistance in the laboratory.

Table 1 LIGNIN COMPOSITION (mg /100g)OF *B.CORIACEA*

Chemicals	Dist. Extract (100°C)	Dist. Extract (200°C)	Dist. Extract (300°C)	Fermented extract	Methanolic Extract	La Casera Soda
Total Lignin	<0.00001	<0.00001	<0.00001	16.94	18.36	<0.00001

Table 2 TANNIC ACID COMPOSITION (mg /100g)OF *B.CORIACEA*

Chemicals	Dist. Extract (100°C)	Dist. Extract (200°C)	Dist. Extract (300°C)	Fermented extract	Methanolic Extract	La Casera Soda
Total tannins	1.19429×10^{-2}	<0.00001	<0.00001	5.37802	5.58378	5.52890×10^{-5}

Table 3 PHYTATE COMPOSITION (mg /100g)OF *B.CORIACEA*

Chemicals	Dist. Extract (100°C)	Dist. Extract (200°C)	Dist. Extract (300°C)	Fermented extract	Methanolic Extract	La Casera Soda
Total Phytate	<0.00001	<0.00001	<0.00001	0.019	0.021	<0.00001

Table 4 OXALATE COMPOSITION (mg /100g)OF *B.CORIACEA*

Chemicals	Dist. Extract (100°C)	Dist. Extract (200°C)	Dist. Extract (300°C)	Fermented extract	Methanolic Extract	La Casera Soda
Total Oxalate	<0.00001	<0.00001	<0.00001	0.012	0.007	0.006

Table 5 PHENOLIC ACIDS COMPOSITION (mg /100g) OF*B.CORIACEA*

Chemicals	Dist. Extract (100°C)	Dist. Extract (200°C)	Dist. Extract (300°C)	Fermented extract	Methanolic Extract	La Casera Soda
Cinnamic Acid	<0.00001	<0.00001	1.16585 x 10-4	1.16756×10^{-4}	6.90109×10^{-4}	<0.00001
Gentisic Acid	<0.00001	<0.00001	1.78440 x 10-4	4.13499×10^{-4}	1.19299×10^{-3}	<0.00001
Protocatechuic Acid	<0.00001	<0.00001	6.81668	21.26729	45.86848	<0.00001
p- coumaric Acid	<0.00001	<0.00001	19.47444	74.69031	135.62324	<0.00001
Vnillic Acid	<0.00001	<0.00001	52.16844	259.93161	240.26866	<0.00001
o-coumaric Acid	<0.00001	<0.00001	37.54758	61.25203	104.62505	<0.00001
p-hydroxybenzoic Acid	<0.00001	<0.00001	37.34104	109.64513	184.87767	<0.00001
Gallic Acid	<0.00001	<0.00001	1.97369	8.81410	14.20961	<0.00001
Caffeic Acid	<0.00001	<0.00001	3.32910	14.77698	23.03777	<0.00001
Ferulic Acid	<0.00001	<0.00001	51.72047	236.65068	228.25001	<0.00001
Syringic Acid	<0.00001	<0.00001	37.95332	103.58682	142.98131	<0.00001
Piperic Acid	<0.00001	<0.00001	2.77715 x 10-1	1.27837	1.78069	<0.00001
Chlorogenic Acid	<0.00001	<0.00001	19.41284	73.98360	127.13449	<0.00001
Rosmarinio	<0.00001	<0.00001	5.96636 x 10-1	2.66580	9.90356	<0.00001
Total	<0.00001	<0.00001	268.61225	968.54326	1258.56241	<0.00001

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Table 6FLAVONOIDS COMPOSITION (mg /100g)OF *B.CORIACEA*

Chemicals	Dist. Extract (100oC)	Dist. Extract (200oC)	Dist. Extract (300oC)	Fermented extract	Methanolic Extract	La Casera Soda
Catechin	<0.00001	<0.00001	<0.00001	5.00×10^{-4}	6.91538×10^{-4}	<0.00001
Resveratrol	<0.00001	<0.00001	<0.00001	8.11×10^{-6}	1.19756×10^{-5}	<0.00001
Genistein	<0.00001	<0.00001	<0.00001	7.80×10^{-3}	1.18105×10^{-2}	<0.00001
Daidzein	<0.00001	<0.00001	<0.00001	8.04×10^{-6}	1.22205×10^{-5}	<0.00001
Apigenin	<0.00001	<0.00001	<0.00001	5.43	8.46392	<0.00001
Daidzein	<0.00001	<0.00001	<0.00001	9.48×10^{-6}	1.45006×10^{-5}	<0.00001
Butein	<0.00001	<0.00001	<0.00001	1.51×10^{-5}	2.41678×10^{-5}	<0.00001
Naringenin	<0.00001	<0.00001	<0.00001	4.33×10^{-2}	6.28597×10^{-2}	<0.00001
Biochanin	<0.00001	<0.00001	<0.00001	2.76×10^{-5}	4.19431×10^{-5}	<0.00001
Luteolin	<0.00001	<0.00001	<0.00001	4.93	7.82451	<0.00001
Kaemferol	<0.00001	<0.00001	<0.00001	18.47	16.96828	<0.00001
(-)-Epicatechin	<0.00001	<0.00001	<0.00001	4.77×10^{-2}	7.84803×10^{-2}	<0.00001
(-) –Epigallocatechin	<0.00001	<0.00001	<0.00001	1.10×10^{-2}	1.78639×10^{-2}	<0.00001
Quercetin	<0.00001	<0.00001	<0.00001	159.32	174.51288	<0.00001
Gallocatechin	<0.00001	<0.00001	<0.00001	1.19	1.95480	<0.00001
(-) – Epicatechin-3-gallate	<0.00001	<0.00001	<0.00001	3.27×10^{-4}	5.24412×10^{-4}	<0.00001
(-) – Epigallocatechin-3-gallate -gallate	<0.00001	<0.00001	<0.00001	2.02×10^{-3}	3.33752×10^{-3}	<0.00001
Isorhamnetin	<0.00001	<0.00001	<0.00001	51.47	82.99467	<0.00001
Robinetin	<0.00001	<0.00001	<0.00001	1.76×10^{-5}	1.50355×10^{-5}	<0.00001
Myricetin	<0.00001	<0.00001	<0.00001	13.08	11.08510	<0.00001
Biacalein	<0.00001	<0.00001	<0.00001	2.41×10^{-4}	3.90104×10^{-4}	<0.00001
Nobiletin	<0.00001	<0.00001	<0.00001	1.51×10^{-2}	2.48491×10^{-2}	<0.00001
Kaempferol-3,7,4'-trimethyl ether	<0.00001	<0.00001	<0.00001	4.94×10^{-3}	7.64335×10^{-3}	<0.00001
Quercetin-3,7,4'-trimethyl ether	<0.00001	<0.00001	<0.00001	15.16	13.62375	<0.00001
Baicalin	<0.00001	<0.00001	<0.00001	1.23×10^{-2}	2.79819×10^{-2}	<0.00001
Tangeretin	<0.00001	<0.00001	<0.00001	4.94×10^{-3}	5.19214×10^{-3}	<0.00001
Hyperoside	<0.00001	<0.00001	<0.00001	3.03×10^{-3}	-	<0.00001
Quercetin-3,7,3',4'-tetramethyl ether	<0.00001	<0.00001	<0.00001	4.20	6.92310	<0.00001
Artemetin	<0.00001	<0.00001	<0.00001	3.61	6.06171	<0.00001
Silymarin	<0.00001	<0.00001	<0.00001	1.02×10^{-2}	1.73722×10^{-2}	<0.00001
Naringin	<0.00001	<0.00001	<0.00001	-	-	<0.00001
Kaempferol-3-arabinoside	<0.00001	<0.00001	<0.00001	3.79	6.06866	<0.00001
Quercitrin	<0.00001	<0.00001	<0.00001	9.04	15.64520	<0.00001
Isoquercetin	<0.00001	<0.00001	<0.00001	22.90	43.57219	<0.00001
Orientin	<0.00001	<0.00001	<0.00001	2.17	4.13105	<0.00001
Rutin	<0.00001	<0.00001	<0.00001	2.12×10^{-3}	4.17676×10^{-3}	<0.00001
Isoorientin	<0.00001	<0.00001	<0.00001	14.87	33.04695	<0.00001
Hesperidin	<0.00001	<0.00001	<0.00001	5.28	10.06283	<0.00001
Total	<0.00001	<0.00001	<0.00001	327.08278	443.20288	<0.00001

Table 7 TERPENES COMPOSITION (mg /100g)OF *B.CORIACEA*

Chemicals	Dist. Extract (100°C)	Dist. Extract (200°C)	Dist. Extract (300°C)	Fermented extract	Methanolic Extract	La Casera Soda
Alpha Pinene	1.34477×10^{-4}	1.00631×10^{-4}	1.04802 x 10-4	1.11103×10^{-4}	1.07563×10^{-4}	<0.00001
Beta Pinene	2.17887×10^{-4}	2.45976×10^{-4}	2.49866 x 10-4	2.51768×10^{-4}	2.50494×10^{-4}	<0.00001
Limonene	1.60375×10^{-3}	8.04828×10^{-4}	9.31493x 10-3	1.37888×10^{-2}	1.09921×10^{-2}	<0.00001
CisOcimene	2.92661×10^{-5}	1.41186×10^{-4}	1.41258x 10-4	1.41296×10^{-4}	1.41276×10^{-4}	<0.00001
Myrcene	8.76946×10^{-5}	1.09738×10^{-4}	1.09936 x 10-4	1.10055×10^{-4}	1.09921×10^{-4}	<0.00001
AlloOcimene	1.03500×10^{-4}	1.41977×10^{-4}	1.42184 x 10-4	1.42441×10^{-4}	1.42237×10^{-4}	<0.00001

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Camphene	6.38115 x 10^{-4}	2.09674 x 10^{-4}	9.30097 x 10-4	1.32701 x 10^{-3}	1.08156 x 10^{-3}	<0.00001
Alpha Thujene	1.10394 x 10^{-4}	1.81407 x 10^{-4}	2.68508 x 10-4	3.17532 x 10^{-4}	2.88646 x 10^{-4}	<0.00001
Neral	2.71858 x 10^{-4}	2.71858 x 10^{-4}	5.74464 x 10-4	7.35850 x 10^{-4}	6.39494 x 10^{-4}	<0.00001
1,8-Cineole	1.09862 x 10^{-3}	9.86342 x 10^{-4}	3.22596 x 10-3	4.58243 x 10^{-3}	3.76506 x 10^{-3}	<0.00001
Borneol	2.35705 x 10^{-4}	1.99378 x 10^{-4}	2.70052 x 10-4	1.33554 x 10^{-2}	2.86792 x 10^{-4}	<0.00001
Nerol(Geraniol)	8.25115 x 10^{-5}	5.61050 x 10^{-5}	5.61064 x 10-5	5.61128 x 10^{-5}	5.61113 x 10^{-5}	<0.00001
Alpha Terpineol	1.14174 x 10^{-4}	2.29051 x 10^{-4}	2.29051 x 10-4	2.29051 x 10^{-4}	2.29051 x 10^{-4}	<0.00001
Terpinen -4-ol	6.36296x 10^{-3}	5.73309 x 10^{-4}	1.77976 x 10-3	2.51097 x 10^{-3}	2.09516x 10^{-3}	<0.00001
Borneol Acetate	8.91640 x 10^{-4}	8.22153 x 10^{-4}	2.36729 x 10-3	3.25795 x 10^{-3}	2.75573 x 10^{-3}	<0.00001
Neryl Acetate	3.13998 x 10^{-4}	2.19361 x 10^{-4}	4.01747 x 10-4	6.07225 x 10^{-1}	6.24015 x 10^{-1}	<0.00001
Geranyl Acetate	1.36885 x 10^{-3}	33.13224 x 10^{-3}	9.16274 x 10-1	8.79763 x 10^{-1}	1.07176	<0.00001
Alpha Amyrin	8.38051 x 10^{-3}	2.88875 x 10^{-3}	26.17537	26.80467	30.77064	<0.00001
Taraxeron	1.37013 x 10^{-4}	2.50387 x 10^{-4}	2.50548 x 10-4	1.17599 x 10^{-4}	2.50586 x 10^{-4}	<0.00001
Triacontane	1.36724 x 10^{-4}	1.64031 x 10^{-4}	8.14885	13.80920	18.30063	<0.00001
Lupeol	2.02698 x 10^{-2}	2.35700 x 10^{-2}	52.29602	72.41663	81.62060	<0.00001
Total	3.68628 x10^{-2}	3.52984 x 10^{-2}	87.55693	114.55852	132.41083	<0.00001

Table 8 PHYTOSTEROLS COMPOSITION (mg /100g) OF *B.CORIACEA*

Chemicals	**Dist. Extract (100°C)**	**Dist. Extract (200°C)**	**Dist. Extract (300°C)**	**Fermented extract**	**Methanolic Extract**	***La Casera Soda***
Cholesterol	<0.00001	<0.00001	<0.00001	5.52859 x 10^{-2}	5.19847 x 10^{-2}	<0.00001
Cholestanol	<0.00001	<0.00001	<0.00001	3.85189 x 10^{-2}	3.83218 x 10^{-2}	<0.00001
Ergosterol	<0.00001	<0.00001	<0.00001	2.03166 x 10^{-3}	1.84777 x 10^{-3}	<0.00001
Campesterol	<0.00001	<0.00001	<0.00001	9.80427 x 10^{-1}	19.66083	<0.00001
Stig –Masterol	<0.00001	<0.00001	<0.00001	9.74391 x 10^{-1}	12.28051	<0.00001
5Avenasterol	<0.00001	<0.00001	<0.00001	6.06790 x 10^{-2}	6.06124 x 10^{-2}	<0.00001
Sitosterol	<0.00001	<0.00001	<0.00001	2.79687	82.27125	<0.00001
Total	<0.00001	<0.00001	<0.00001	4.90820	114.36536	<0.00001

Table 9 Total phytochemical profile (mg/100g) in study samples

Chemicals	Raw	Fermented	Methanolic	100°C distillate	200°C distillate	300°C distillate	Soda water
Flavonoids	96	327.08	443.20	<0.00001	<0.00001	<0.00001	<0.00001
Phenolic Acid	688	968.54	1258.56	<0.00001	<0.00001	268.61	<0.00001
Tannic Acid	10	5.38	5.58	1.19 x 10^{-2}	<0.00001	<0.00001	5.53 x 10^{-5}
Phytosterol	4	4.91	114.37	<0.00001	<0.00001	<0.00001	<0.00001
Terpenes	80	114.56	132.41	3.69 x 10^{-2}	114.56	114.56	<0.00001
Phytate	0.11	0.02	0.02	<0.00001	<0.00001	<0.00001	<0.00001
Oxalate	0.07	0.01	0.01	<0.00001	<0.00001	<0.00001	0.01
Lignin	20.2	16.94	18.36	<0.00001	<0.00001	<0.00001	<0.00001
Total	898.38	1437.44	1972.51	4.88 x 10^{-2}	114.56	383.17	0.01

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

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