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IN VITRO GASTRO-PROTECTIVE EFFECT AND BIOACTIVE COMPOUNDS FROM TERMINALIA BELLERICA ROXB. SEEDS

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ABSTRACT

Aim: Terminalia bellerica represents an important medicinal plant used to treat a variety of ailments including inflammation, however there appears little scientific evidence to support its therapeutic efficacy. The present study deals with in vitro gastro-protective effect of an ethanolic seed extract from Terminalia bellerica with special reference to inflammatory bowel diseases which are characterized by perpetual intestinal inflammation, oxidative stress and an increased propensity to develop colon cancer. Materials and Methods: The antioxidant activity was assessed using DPPH radical scavenging assay, the extract was measured by its capacity for Inhibition of Nitric Oxide (NO) production in lipopolysaccharide (LPS) treated macrophages and cytotoxicity of the extract was measured in HT-29 and Vero cells. The extract was phytochemical screened and also the bioactive compounds were isolated and identified by different spectroscopic tools (UV, 1H-NMR, 13C-NMR, MS). Results: EtOH extract of Terminalia bellerica seeds proved a significant antioxidant capacity with an EC50 value of 0.86 µg/ml. and T. bellerica extract significantly inhibited nitric oxide production in LPS stimulated macrophages. The extract was essentially non-cytotoxic toward Vero cells, but inhibited HT-29 cell growth as judged from MTT viability assays and colony forming capacity. Phytochemical investigation of T. bellerica seeds EtOH extract showed that it contained different bioactive constituents as flavonoids, terpenes, carbohydrates, coumarins and tannins, further phytochemical analysis resulted in the isolation and identification of β-sitosterol, gallic acid, vanilic acid, ellagic acid, scopoletin, quercetin, apignin 7-O-β-glucoside, quercetin 3-O-α-rhamnoside and quercetin 3-O-β-glucoside. Conclusion: Seeds of T. bellerica EtOH extract contained number of known anti-inflammatory and antioxidant compounds which can explain the in vitro gastro-protective properties observed and provide the first verification as to the therapeutic potential of this plant for the treatment of gastrointestinal diseases especially inflammatory bowel diseases.

Keywords: ***Terminalia bellerica, seeds, gastro-protective, antioxidant, anti-inflammatory, IBD.***

INTRODUCTION

Ulcerative colitis and Crohn's disease, collectively termed inflammatory bowel diseases (IBD), represent chronic inflammatory disorders of the gastrointestinal tract. The major pathogenic feature of IBD is the development of a dysfunctional epithelial barrier and consequent immune responses directed against the normal enteric microflora which are predominantly mediated by activated neutrophils, monocytes and macrophages with the concomitant formation of reactive oxygen and nitrogen radicals [1]. Chronic inflammation and oxidative stress occurring in IBD patients have been proposed to promote the long term development of colon cancer, a characteristic feature strongly associated with IBD [2]. Plants are considered by many to be one of the most promising therapeutic sources due to their variety of species and applications. Plants have been shown to contain many phytochemicals with various bioactivities, including, anticancer, antioxidant and anti-inflammatory activities. In recent years, natural products have attracted the interest of many researchers as a potential and useful source for antitumor, antioxidant and anti-inflammatory agents. *Terminalia bellerica* Roxb. a large deciduous tree, found throughout Central Asia and some other parts of the world. It belongs to *combretaceae* family and it is commonly known as belleric myrobalan. In traditional medicine, *T. bellerica* fruits are used to treat asthma, cancer, colic, diarrhea, dysuria, headache, hypertension, inflammation and pain [3, 4]. The plant is reported to contain termilignan, gallic, ellagic and belleric acids, flavonoids and tannins. *T. bellerica* possess antioxidant, antispasmodic, antibacterial, cardioprotective, hepatoprotective, hypoglycemic, and hypotensive properties [5, 6, 7]. It is also one of the constituents of "Triphala" which is prescribed as a treatment for diseases of the liver and gastrointestinal tract [8, 9], however there appears to be little scientific evidence confirming the gastro-protective properties of *T. bellerica* and its potential to treat gastrointestinal disorders such as IBD. In the present study we have established that *T. bellerica* seeds extract possess medicinal properties well suited to the treatment of IBD both in terms of biological activity and the presence of known anti-inflammatory and antioxidant bioactive compounds.

Materials and Methods:

Experimental

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). Spectroscopic data: NMR–Varian. MS (Finnigan MAT SSQ 7000, 70 ev). Silica gel (60-200 mesh, Merck) for column chromatography, Thin Layer Chromatography (TLC): pre-coated sheets of silica gel 60 F_{254} (Merck). Sephadex LH-20 (Sigma).

Plant material

T. bellerica seeds were collected from the Zoo garden, Giza, Egypt on June 2011 and the plant identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Zoo garden, Giza, Egypt.

Preparation of the extract

Finely ground *T. bellerica* seeds 750 g were extracted with ethanol 95%. The extract was concentrated to dryness in vacuo at 50^0C to give 45 g of crude extract. The extract was tested for the presence of bioactive compounds by using standard tests (Molisch 's test for carbohydrates, Shinoda test for flavonoids, forth the test for saponins, Salkowski 's for terpenes and sterols, $FeCl_3$ and Mayer's reagents for detecting of tannins and alkaloids, respectively [10, 11, 12].

Preparation of extracts for biological assays

Dried extract was reconstituted in DMSO to a concentration of 100µg/µl and then diluted with complete medium to the concentrations indicated. The final DMSO content in the assay is maintained below 1%, a concentration which has minimal effect on cell viability.

Biological assays:

Inhibition of NO production in LPS treated macrophages

Murine peritoneal macrophage cells (RAW264.7) were cultured in DMEM containing 10% fetal bovine serum (FBS) (LONZA). Cells were seeded into 96 well plates at a density of 8 X 10^4 cells/well and allowed to attach overnight. The cells were then treated with 1µg/ml LPS (SIGMA) and the indicated concentrations of test sample for an additional 18hr. To measure nitrate levels, 50µl of the spent culture medium was removed and added to an equal volume of Griess reagent (SIGMA). The absorbance was measured at 540nm using a microplate reader and the nitrate concentrations were calculated by comparison with the absorbance to sodium nitrate standard solutions. Aminogaunidine (SIGMA) was used as a positive control to demonstrate the inhibition of nitrate production. Cell viability was simultaneously measured using the standard MTT viability assay.

DPPH radical scavenging activity

The extract was diluted in ethanol/water (1:1) from 10mg/100µl stocks prepared in DMSO. Five µl samples were placed into each well of a 96-well plate and then a 120µl of Tris-HCl buffer (50mM, pH7.4) was added followed by 120µl of freshly prepared DPPH solution (0.1mM in ethanol). The plate was incubated for 20min at room temperature and the absorbance read at 513 nm. Percentage of DPPH scavenging was calculated as [(A- B/A) × 100] where A represents absorbance without test samples, and B represents absorbance in the presence of test samples. Ascorbic acid

was used as a positive control (EC_{50} = 24.07µg/ml).

Cytotoxicity in HT-29 and Vero cells

HT-29 cells were seeded into 96-well culture plates at 6000 cells/well in DMEM supplemented with 10% FBS and left for 24 hours. Various dilutions of the extract were added and the cells incubated for a further 48 hrs after which the medium was replaced with 200 µl MTT (Sigma) (0.5 mg/ml in DMEM). After further 2 hr incubation at 37°C, the MTT was removed and the purple formazan product dissolved in 200µl DMSO. The absorbance was measured at 560 nm using a multiwell scanning spectrophotometer (Multiscan MS, Labsystems). All incubation steps were carried out in a 37°C humidified incubator with 5% CO_2. Vero cells were treated identical to that of HT-29, except the cells were seeded at a density of 10 000 cells per well.

Colony forming assay using HT-29 cells

HT-29 cells were seeded into 6-well plates at a density of 250 cells per well and allowed to attach overnight. Cells were then treated with the indicated concentrations of plant extract or positive control (melphalan) for an additional 7 days. Colonies were visualized by staining with 1% crystal violet for 10min followed by two brief washes with distilled water.

Isolation of bioactive compounds from ethanol extract of *T. bellerica* seeds

The extract 45 g was fractionated on silica gel column chromatography, and eluted with n-hexane, chloroform, ethyl acetate and methanol gradually. Four main fractions were collected. Fraction 1 (1.2 g) was subjected to further fractionation on sub-column of silica gel eluted with n-hexane-chloroform (50:50) to give the compound 1 (β-sitosterol) and further elution with chloroform : ethyl acetate (50:50) gave compound 2 (Gallic acid). Fraction 2 (1.65 g) was subjected to further fractionation on sub-column of silica gel eluted with chloroform: EtOAc (70:30) gave compound 3 (vanilic acid) and further elution with EtOAc gave compound 4 (ellagic acid). Fraction 3 (940 mg) was subjected to further fractionation on sub-column of silica gel eluted with EtOAc: MeOH (98:2) gave compound 5 (scopoletin) and elution with EtOAc: MeOH (96:2) gave compound 6 (quercetin) and elution with EtOAc: MeOH (90:10) gave compound 7 (apigenin 7-O-β-glucoside). Also fraction 4 (1.95 g) was subjected to further fractionation on sub-column of silica gel eluted with EtOAc: MeOH (85:15) gave compound 8 (quercetin 3-O-α-rhamnoside) and further elution gave compound 9 (quercetin 3-O-β-glucoside). All the compounds were purified on sephadex LH–20 column.

Acid hydrolysis of flavonoid glycosides: Solutions of 5 mg of compounds 7, 8 and 9 in 5 ml 10% HCl was heated for 5h. The aglycones were extracted with EtOAc and identified by co-TLC with authentic standards.

The sugars in the aqueous layer were identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in a solvent system (*n*-BuOH-AcOH-H_2O 4:1:5 upper layer).

Results:

Antioxidant activity

DPPH radical scavenging activity compared very favorably to that of ascorbic acid. The extract has shown a significant antioxidant activity (EC_{50} value of 0.86 µg/ml) compared with ascorbic acid (EC_{50} value of 24.07µg/ml).

Anti-inflammatory activity

Significant inhibition of nitrate production was evident in *T bellerica* treated RAW 264.7 cells however in the absence of LPS there was no stimulation of nitrate production, indicating that the extract does not possess pro-inflammatory properties (Fig. 1).

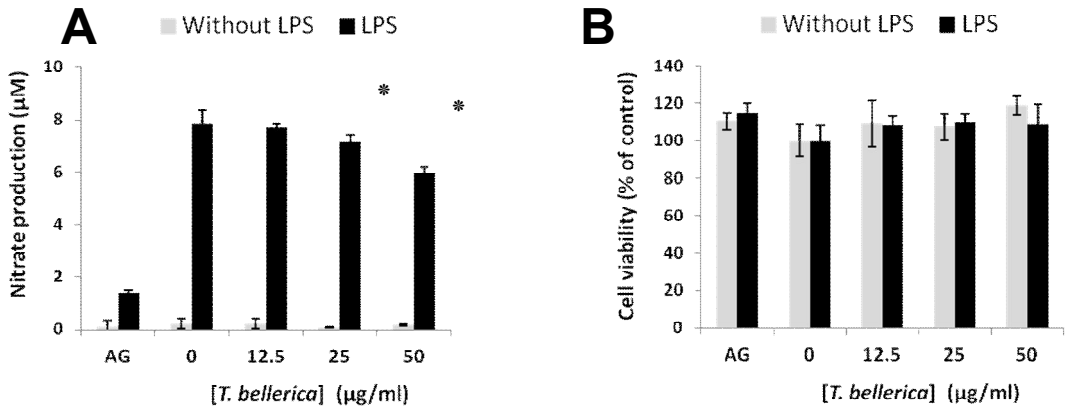


Figure 1: **Nitrate production in mouse macrophages (RAW 264.7) treated with crude extract.**

Cells were incubated for 18hr in the presence or absence of bacterial LPS (1µg/ml) and two concentrations of test extract. The cell culture medium was assayed for nitrate using Griess reagent. Cell viability in the presence of corresponding concentrations was assessed by the MTT reduction assay. The data represents the mean ± STDev of four replicates from two independent experiments. Significant ($p<0.05$) reductions in the levels of nitrate are indicated as *.

Cytotoxicity

T. bellerica extract did not significantly affect the viability of Vero cells at concentrations below 500µg/ml (Fig 2A). In contrast, proliferating HT-29 cells were significantly inhibited at 100µg/ml; however the inhibitory effect appears saturated above 50% viability suggesting that the extract is cytostatic as opposed to cytotoxic (Fig. 2B). Colony forming assay confirmed the cytostatic nature in that at a concentration of 100µg/ml cell growth was completely inhibited but individual cells were still visible after 7 days of continuous treatment (Fig. 2C). Lower concentrations did

not reduce the number of colonies formed although the size of the colonies did appear smaller.

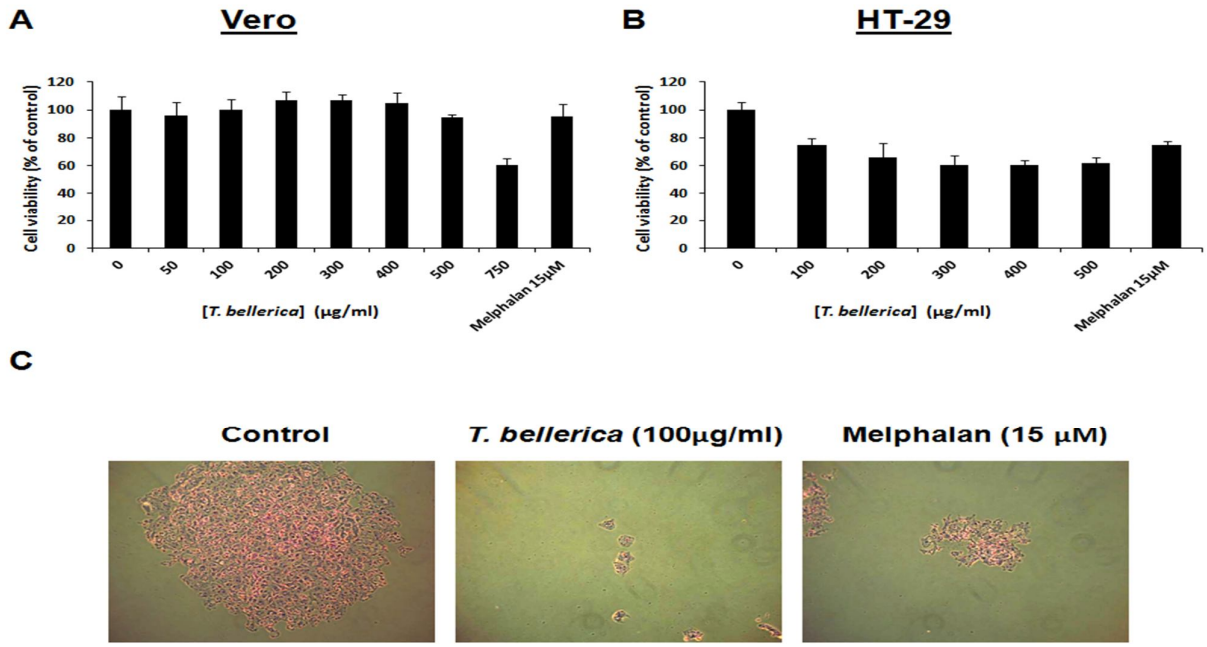


Figure. 2: Cytotoxicity of *T. bellerica* extract in normal versus cancer cells. A and B) Dose dependent effects of *T. bellerica* seeds on the viability of Vero and HT-29 cells respectively. Each data point represents the mean±STDev for quadruplicate assays. C) Colony forming capacity of HT-29 treated with *T. bellerica* extract or malphalan.

Phytochemical analysis

Results of phytochemical analysis of *T. bellerica* seeds EtOH extract is compiled in Table 1. The major bioactive components of *T. bellerica* seeds EtOH extract are β-sitosterol, gallic acid, ellagic acid, vanilic acid, scopoletin, quercetin, apignin 7-O-β-glucoside, quercetin 3-O-α-rhamnoside and quercetin 3-O-β-glucoside. All the structures of the bioactive compounds were proved by different spectroscopic tools. The chemical structures of the compounds are shown in Fig. 3.

Table 1: Phytochemical analysis of *T. bellerica* seeds EtOH extract

Phytoconstituents	*T. bellerica* EtOH extract
Triterpenes and /or Sterols	+
Carbohydrates and/or glycosides	+
Flavonoids	+
Coumarins	+
Alkaloids and/or nitrogenous compounds	-
Tannins	+
Saponins	-

(+) presence of constituents, (-) absence of constituents

Structure Elucidation of the bioactive compounds

β-sitosterol (1): 15 mg, white needles, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.37 (IH, m, H-6), 3.52 (IH, m, H-3), 1.09 (3H, s, CH3-19), 0.98 (3H, d, J= 6.5, CH3-21), 0.92 (3H, t, J= 7.4, CH3-29), 0.85 (3H, d, J= 6.7Hz, CH3-26), 0.81 (3H, d, *J*= 6.7Hz, CH3-27), 0.75 (3H, s, CH3-18). ^{13}C-NMR(100 MHz, $CDCl_3$): δ 140.4 (C-5), 121.5 (C-6), 71.6 (C-3), 57.2 (C-17), 56.4 (C-14), 50.3 (C-9), 46.3 (C-24), 42,8 (C-13, 4), 39.8 (C-12), 37.6 (C-1), 36.7 (C-10), 35.9 (C-20), 34.2 (C-22), 31.7 (C-8, 7), 31.4 (C-2), 29.2 (C-25), 28.4 (C-16), 26.2 (C-23), 24.5 (C-15), 23.4 (C-28), 21.1 (C-11), 19.8 (C-26), 19.5 (C-19), 19.2 (C-27), 18.6 (C-21).

Gallic acid (2), 23 mg: White amorphous powder. ^{1}H-NMR (DMSO-d6, 400 MHz): d 7.15 (2H, s, H-2,6). ^{13}C-NMR (DMSO-d6, 100 MHz): d 167.25 (-COOH), 145.75 (C-3,5), 137.72 (C-4), 121.23 (C-1), 109.16 (C-2,6).

Vanilic acid (3): 12 mg, white powder, λ_{max} (nm), MeOH: 256, 294 (+NaOMe) 283, 298. ^{1}H-NMR (270 MHz, DMSO-d_6): δ ppm 8.87 (1H,s, OH carboxylic), 8.52 (1H, s, OH-4) 7.56 (1H, s, H-2), 7.12 (2H,dd, *J*=2.5, *J*= 9.2, H-6), 7.08 (1H, d, J = 9.2 , H-5), 3.69 (3H, s, OCH_3).

Ellagic acid (4), 32 mg, white amorphous powder. ^{1}H-NMR (DMSO-d6,400 MHz): d 7.44 (2H, s, H-4,9). 13C-NMR (DMSO-d6, 100 MHz): d 158.8 (5,10-CO),147.8 (C 3,8), 139.3 (C-2,7), 136.1 (C-1a,6a), 112 (C-4b,9b), 110.2 (C-4,9), 107.3 (4a,9a).

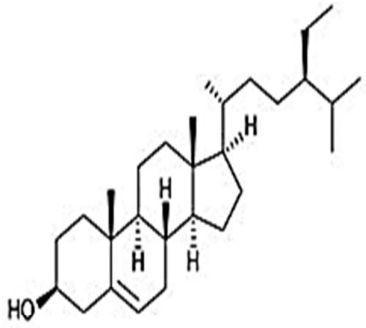
Scopoletin (5): 18 mg, colourless needles. 1H-NMR (CDCl3,400 MHz): δ ppm 6.23 (1H, d, J=9.4, H-3), 7.62 (1H, d, J=9.4, H-4), 7.38 (1H, s, H-5), 3.91 (3H, s, OCH3), 6.81 (1H, d, s, H-8), EI-MS: *m/z* 192.

Quercetin (6): 8 mg, yellow powder. UV λmax (MeOH): 255, 267, 371; (NaOMe): 270, 320, 420; ($AlCl_3$): 270, 455; ($AlCl_3$/HCl): 264, 303sh, 315sh, 428; (NaOAc): 257, 274, 318, 383; (NaOAc/H_3BO_3): 259, 387. EI-MS: m/z 302.

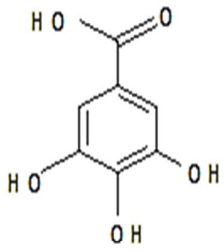
Apigenin 7-*O*-*β*-glucoside (7): 19 mg, yellow crystals. ^{1}H-NMR (DMSO, 270 MHz): δ ppm 12.95 (1H, s, 5-OH), 7.95 (2H, d, *J* = 8.5 Hz, H-2´,6´), 6.95 (2H, d, *J* = 8.5 Hz,H-3´,5´), 6.85 (1H, s, H-3), 6.8 (1H, d, *J* = 2Hz, H-8), 6.45 (1H, d, *J* =2 Hz, H-6), 5.04 (1H, d, *J* = 7.2 Hz, H-1´´). ^{13}C-NMR (DMSO-d_6, 100 MHz) : δ 182.28 (C-4), 164.42 (C-2), 163.28 (C-7), 162.12 (C-5), 161.65 (C-4'), 157.47 (C-9), 129.16 (C-2',6*), 121.22 (C-r), 116.68 (C-3',5'), 105.84 (C-10), 103.42 (C-3), 100.47 (C-1"), 100.32 (C-6), 95.35 (C-8), 77.74 (C-5"), 76.98 (C-3"), 73.63 (C-2"), 70.14 (C-4"), 61.15 (C-6").

Quercetin 3–O–α–rhamnoside (8): 24 mg, yellow crystals: ^{1}H–NMR (400 MHz, DMSO–d6) δ ppm 7.26 (2H, m, H–2`, 6`), 6.83 (1H, d, J=9 Hz, H–5'), 6.49 (1H,d, *J*=2.5 Hz, H–8),6.14(1H, d, J=2.5Hz, H–6), 5.25 (1H, br s, H–1'') 0.78 (3H, d, *J*=6Hz). ^{13}C–NMR (100 MHz, DMSO–d6): δ ppm 177.42 (C–4), 167.45 (C–7), 161.40 (C–5), 157.01 (C–2), 157.45 (C–9), 149.19 (C–4`), 145.57 (C–3`), 134.12 (C–3), 131.97 (C–6`), 121.40 (C–1`), 115.71 (C–2`), 115.40 (C–5`), 103.10 (C–10), 101.97 (C–1``), 99.98 (C–6), 94.47 (C–8), 71.47 (C–4''), 70.94, 70.85, 70.62 (C–2``, C–5``, C–3''), 17.78 (C6'').

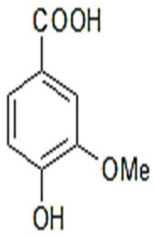
Quercetin 3-O-β-glucoside (9): 21 mg, yellow amorphous powder: ^{1}H-NMR (400 MHz, DMSO-d6), δ ppm 7.58 (2H, m, H-2`/6`), 6.83 (1H, d, *J* = 9 Hz, H-5'), 6.35 (1H,d, *J* = 2.5 Hz, H-8), 6.15 (1H,d, *J*=2.5Hz, H-6), 5.46 (1H, d, *J* =7.55 Hz, H-1``), 3.2-3.8 (m, sugar protons). ^{13}C-NMR (100 MHz, DMSO-d6): δ ppm 177.83 (C-4), 162.14(C-7), 161.73 (C-5), 157.38 (C-2), 156.89(C-9), 149.11 (C-3`), 148.47(C-4'), 145.38(C-5`), 133.77(C-3), 121.61 (C-1`), 116.65(C-2') 115.72(6'), 104.21 (C-10), 101.44 (C-1''), 99.37 (C-6), 94.12 (C-8), 78.08 (C-5``), 77.03 (C-3``), 74.62 (C-2``), 70.45(C-4'') 61.48 (C-6``).



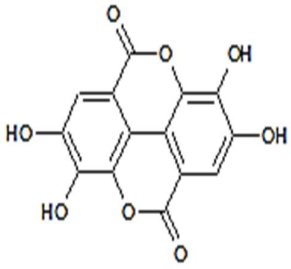
Compound 1: β-Sitosterol



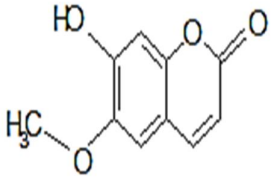
Compound 2: Gallic acid



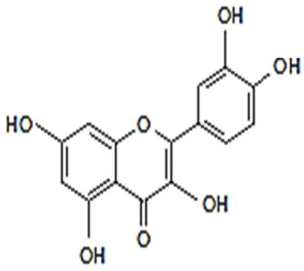
Compound 3: Vanilic acid



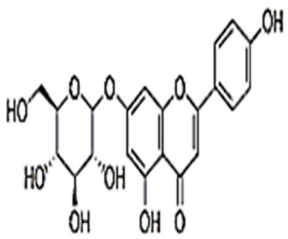
Compound 4: Ellagic acid



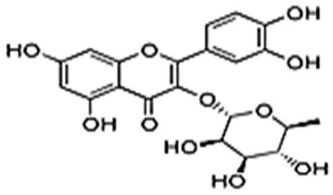
Compound 5: Scopoletin



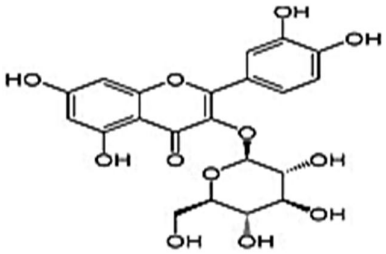
Compound 6: Quercetin



Compound 7: Apigenin 7-O-β-glucoside



Compound 8: Quercetin 3-O-α-rhamnoside



Compound 9: Quercetin 3-O-β-glucoside

Fig. 3. Compounds isolated from *T. bellerica* seeds EtOH extract

Discussion:

Chromatographic separation and purification of EtOH from *Terminalia bellerica* seeds resulted in the isolation and identification of nine compounds, β-sitosterol, gallic, ellagic and vanilic acids, scopoletin, quercetin, apignin 7-O-β-glucoside, quercetin 3-O-α-rhamnoside and quercetin 3-O-β-glucoside. Compound 1 (β-sitosterol) gave a dark spot under short UV light and also gave a violet color after spraying with vanillin sulphuric and heating in an oven at 110°C for 5min. NMR spectral data have shown signals very close to compound 1 [13, 14] and confirmation was done by co-TLC with authentic standards. Compound 2 (Gallic acid) gave a violet spot under short UV light and gave a specific dark green color with $FeCl_3$, NMR data are in accordance with the published literature [15]. Compound 3 (vanilic acid) has shown a blue spot on TLC and spectroscopic data was in agreement with vanilic acid [16]. Ellagic acid (compound 4) gave a shiny spot under short UV light and it gave a bluish green color with $FeCl_3$ and this indicates that it is a phenolic compound, NMR data were in agreement with the published literature [17]. Compound 5 (Scopoletin) is a simple coumarin and it gave a blue spot on TLC and after spraying with 10% KOH, it gave yellow green color which is very specific for coumarins and also the spectroscopic data with in accordance with the literature [18]. Compound 6 (quercetin) aglycone showed yellow spots and the two compounds gave fluorescence yellow color after spraying with $AlCl_3$ [19]. Compounds 7, 8 and 9 have shown deep purple spot for each compound under UV light and changed into yellow color when exposed to ammonia vapor, and gave fluorescence yellow after

spraying with $AlCl_3$ [19]. Acid hydrolysis of each compound gave apigenin aglycone and glucose sugar for compound 7, quercetin aglycone and rhamnose sugar for compound 8, while compound 9 gave quercetin aglycone and glucose sugar, respectively. Spectroscopic data for the compounds 7, 8 and 9 are in agreement with compound 7 as apigenin 7-O-β-glucoside, compound 8 is quercetin 3-O-α-rhmanoside and compound 9 is quercetin 3-O-β-glucoside [20]. Since inflammation is intimately related to the production of free radicals, oxidative stress has been proposed as a mechanism underlying the development of IBD and treatment with antioxidants appear to be a viable therapeutic strategy to treat IBD (1) Furthermore, oxidative stress may also exacerbate the onset of colorectal cancer in IBD patients due to DNA damage [21]. Antioxidant capacity of *T. bellerica* seed extract, as determined using the DPPH radical scavenging assay, indicates an exceptional antioxidant activity with an IC_{50} value of 0.86µg/ml, almost 30 fold more potent than ascorbic acid. Although previous studies have shown that *T. bellerica* fruit extracts have high antioxidant activity, 3.6-fold more active than ascorbic acid, our results suggest that the seed extract may be a better source of antioxidants [22]. The presence of compounds such as gallic, ellagic and vanilic acids, quercetin, apignin 7-O-β-glucoside, quercetin 3-O-α-rhamnoside and quercetin 3-O-β-glucoside all provide potential chemical structures to explain the antioxidant activity of the extract [23]. The cellular response to LPS in macrophage cells is transmitted via the Toll-like receptor complex situated in the plasma membrane, which in turn activates a number of signal transduction pathways including NF-κβ which also regulates iNOS expression and concomitant NO production. In the absence of LPS, *T. bellerica* extract exerted no proinflammatory activity as is evident from the lack of NO production (Fig. 1). In contrast, there was a significant decrease in the NO production when RAW macrophages were treated with *T. bellerica* extract, an effect that is independent of cell death as full viability was maintained at the concentrations tested. The presence of the plant steroid β-sitosterol, a known inhibitor of NF-κβ activation, may at least in part explain the decreased NO production [24]. In addition it has been demonstrated that quercetin inhibits both cytokine and iNOS expression through the inhibition of the NF-κβ pathway [25]. It is also of interest to note that flavanoid glycosides are not well absorbed in the upper segments of the intestine and may thus be more likely to reach the colon where they would be hydrolyzed by the intestinal microbiota, releasing the aglycone at the inflamed colon. Considering the presence of apigenin 7-O-β-glucoside, quercetin 3-O-α-rhamnoside and quercetin 3-O-β-glucoside, it is likely that the in vitro anti-inflammatory activity measured in the present study may be further enhanced in the in vivo situation. The Vero cell line is an immortal, non-tumorigenic fibroblastic cell line established from the kidney of African green monkey

(Cercopithecus aethiops) and is extensively used as a normal cell model for toxicity testing and evaluating chemical induced transformation. *T. bellerica* extract revealed little cytotoxicity against Vero cells, supporting the non-toxic nature previously reported for the fruit extract in an *in vivo* model [26]. The extract was however considerably more inhibitory towards the colon cancer cell line HT-29 by significantly reducing the number of viable cells after 48hr treatment with 100µg/ml. Chang liver cells (a suspected HeLa (cervical cancer) derivative) were similarly more sensitive than Vero cells displaying an EC_{50} value of 137µg/ml. Colony forming assays using HT-29 cells revealed that concentrations below 100µg/ml reduced colony formation capacity however cell viability was not significantly altered as judged from the number of colonies formed, suggesting the extract is cytotstatic rather than cytotoxic, at least at concentrations below 100µg/ml. Together with the anti-inflammatory and antioxidant properties which may attenuate inflammation driven carcinogenesis, anti-proliferatory activity of *T. bellerica* seed extract can potentially further reduce the onset of IBD related colon cancer.

Conclusion:

T. bellerica seeds EtOH extract has shown a favorable cytotoxicity profile, antioxidant and anti-inflammatory activities as well as the potential to inhibit colon cancer cell proliferation. These activities can at least in part be explained due to the presence of known bioactive chemical constituents including, carbohydrates, tannins, flavonoids, coumarins and triterpenes. Taken together these findings support the use of T. bellerica as a gastro-protective agent and suggest that the seed extract has therapeutic properties well suited to the treatment of IBD.

Conflict of interest:

There is no conflict of interest associated with the authors of this paper.

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