

## ***In vitro* and *In vivo* Investigations Provide New Insights into Bioactivities of *Blumea clarkei* Hook.f. Leaves**

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### **Abstract**

As there is a resurgence of interest in plants as promising sources of new active pharmaceutical constituents, the present study has been designed to establish the preliminary biological activities of *Blumea clarkei* Hook.f. leaves. Here, the crude methanol extract of the leaves along with its organic and aqueous soluble fractions were subjected to different *in vitro* and *in vivo* assays. The ethyl acetate soluble fraction exhibited the highest total phenolic content (71.59 mg of GAE/gm of extract) and DPPH free radical scavenging (IC<sub>50</sub> value 19.25 µg/ml) whereas another polar fraction; the aqueous soluble extractive showed the maximum cytotoxicity (LC<sub>50</sub> value of 18.60 µg/ml) in the brine shrimp lethality assay. In membrane stabilizing activity evaluation, the inhibition of hypotonic solution and heat induced hemolysis were revealed maximum by the DCM (30.64%) and aqueous soluble fraction (39.15%), respectively while the crude methanol extract exhibited the highest thrombolytic potential (30.47% clot lysis). The central analgesic activity of the crude extract significantly increased the latent response time in tail-immersion method (p<0.001) at doses 200 and 400 mg/kg of body weight after 90 minutes of administration when compared to the control group. In acetic acid-induced writhing method, the plant extract showed prominent peripheral analgesia (p<0.001) with 47.54% and 65.57% inhibition at 200- and 400-mg/kg of body weight, respectively. In addition, a dose dependent hypoglycemic and antidiarrheal activities were also observed by the crude extract.

**Key words:** *Blumea clarkei*, antioxidant, DPPH, cytotoxicity, thrombolytic, analgesic, hypoglycemic, antidiarrheal

### **Introduction**

Fossil records proved plants have been a valuable source of medicine for at least 60 thousand years (Fabricant, 2001; Solecki, 1975). At present, 50% of all approved drugs are sourced from natural product and their derivatives while approximately 25% of the natural products are derived from higher plants (Maridass and De Britto, 2008). Though there has been slightly declined interest in the field of natural product due to the recent advancement of combinatorial chemistry in drug discovery and development (Calixto, 2005), nature is still being considered an invaluable source of immense chemical diversity (Alam *et al.*, 2020) and fragments

aiding the design of new lead molecules (Mortenson *et al.*, 2018; Yñiguez-Gutierrez and Bachmann, 2019). Additionally, only 6% of the plants worldwide have been biologically screened (Verpoorte, 2000). Thus, the demand for systematic investigations of unexplored medicinal plants with potential bioactivities is increasing to instigate the discovery of a plethora of promising drug candidates.

*Blumea clarkei* Hook.f. (Family: Asteraceae; synonyms: *Blumea malabarica* Hook.f., *Blumea lessingii* Merr., *Blumea hongkongensis* Vaniot; Bengali names: monchoytta, toragach-Chakma tribe) is a medicinally eminent perennial herb with obovate or oblanceolate leaves and fibrous rootstock

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("Blumeaclarkei Hook.f.," 2013; Rahman *et al.*, 2007). It is native to tropical and subtropical regions of South and East Asia — India, Myanmar, China, Philippines, and Bangladesh ("Blumeaclarkei Hook.f.," 2013). The species of this genus have long been a valuable part of Unani, *Ayurveda*, homeopathy system of medicine (Jahan *et al.*, 2014) and their folkloric uses include arthritis, fever, infective hepatitis, respiratory disorders i.e. asthmatic bronchitis, and various other diseases (Do *et al.*, 2004). Moreover, a range of pharmacological activities such as antibacterial, anti-catarrhal, anti-inflammatory (Chu *et al.*, 2013), analgesic, diuretic, hypothermic (Pal, 1972), cytotoxic (Jahan *et al.*, 2014), etc. has also been reported for different parts of these plants. In Bangladesh throughout various tribal communities, ubiquitous use of *B. clarkei* leaves' paste to treat jaundice and fractured bone is noticeable (Rahman *et al.*, 2007). Earlier investigations of this genus have reported abundant volatile and non-volatile phytoconstituents including campesterol, diterpenes, flavonoids, sesquiterpenes, and flavones (leaves), cineol, fenchone and blumea camphor (essential oil), triterpenes and sterols (root and root bark), hentriacontane,  $\alpha$ -amyrin, hentriacontanol, and lupeol (aerial part) (Chen *et al.*, 2009; Guan *et al.*, 2012; Jahan *et al.*, 2014). In spite of the broad uses of the plant through the ethnopharmacological spectrum, the phytochemical and pharmacological properties of *B. clarkei* have not been comprehensively assayed to justify its traditional uses. Alongside, as the variety of ecosystem largely affects the phytochemical composition, the bioactivities of the plant are needed to be evaluated based on its Bangladeshi origin. In this view, as part of our continuing investigations of medicinal plants from Bangladesh (Soma *et al.*, 2020; Ahmed *et al.*, 2019; Rashid *et al.*, 2016), the current study aims to preliminary assess the antioxidant, cytotoxic, membrane stabilizing, thrombolytic, analgesic, hypoglycemic and antidiarrheal properties of leaves of *B. clarkei*.

## Materials and Methods

**Collection of plant material and extraction:** The leaves of *B. clarkei* were collected from the remote hilly forest of Chittagong and identified by expert taxonomist and botanist. After proper washing, the leaves were sun-dried for several days followed by pulverization using high capacity grinding machine. The powdered material (500 gm) was soaked in methanol (2 liters) in a clean, amber-colored bottle (3 liters) for 15 days with occasional agitation. The whole mixture was then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The resultant filtrate was then concentrated using Buchii Rota evaporator at low temperature and pressure and the crude extract was weighed properly. Aliquot of the concentrated gummy mass of crude extract (5 gm) was fractionated using a solvent-solvent partitioning method, modified Kupchan Partition (VanWagenen *et al.*, 1993) using three different organic solvents and distilled water. The resulting fractions (pet-ether soluble fraction PESF: 2.45 gm, DCM soluble fraction DCMSF: 2.36 gm, ethyl acetate soluble fraction EASF: 1.20 gm, aqueous soluble fraction AQSF: 1.50 gm) of the total crude extract (MESF) were evaporated to dryness and refrigerated for further analysis.

**Drugs and chemicals:** All reagents used in the experiments were of analytical grade and procured from reliable sources. Tween-80 (BDH Chemicals, UK), acetic acid (Merck, Germany), DMSO Merck (Germany), normal saline solution (Beximco Infusion Ltd., Bangladesh), acetylsalicylic acid, streptokinase (Incepta Pharmaceuticals Ltd., Bangladesh), diclofenac sodium (Incepta Pharmaceuticals Ltd., Bangladesh), loperamide (Opsonin Pharma Ltd., Bangladesh), glibenclamide (Square Pharmaceutical Ltd., Bangladesh) were used in the present study. Besides, according to the previously stated procurement process, morphine was purchased from Gonoshasthaya Pharmaceuticals Limited to conduct the screening for central analgesic activity (Soma *et al.*, 2020).

**Experimental animals:** Both sex of Swiss-albino mice (aged 4-5 weeks, avg. weight 25-30 gm) were collected from the animal branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (icddr,b) which were kept in polypropylene cages in a controlled room (relative humidity 60-70%; temperature  $24 \pm 2^\circ\text{C}$ ; 12hr light-dark cycle) and given icddr,b formulated rodent food and water *ad libitum*. To habituate with the surroundings, they were kept for a week in the experimental environment before starting the test. The principle and recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) were mimicked during the study.

**Grouping of mice:** Randomly four groups were formed consisting of three mice in each group for screening every biological activity. Those groups were named as the positive control (STD), negative control (CTL), and two test groups. The test groups were given the leaves extract at doses of 200 (BC 200) and 400 (BC 400) mg/kg of body weight.

**Total phenolic content:** The quantification of the total phenolic content of the extractives was performed using Folin-Ciocalteu reagent as oxidizing agent and gallic acid as the reference standard (Harbertson and Spayd, 2006). The phenolic contents were expressed as milligrams of gallic acid equivalent (GAE)/ gram of extract.

**DPPH free radical scavenging activity:** Following the method devised by Brand-Williams

*et al.*, the antioxidant activity of the fractions was assayed by neutralizing 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical while antioxidant butylated hydroxyl toluene (BHT) and ascorbic acid (AA) were utilized as standard (Brand-Williams *et al.*, 1995).

% Inhibition of free radical DPPH

$$= \left( 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of the control reaction}} \right) \times 100$$

**Brine shrimp lethality assay:** The preliminary cytotoxicity of extractives on brine shrimp nauplii (*Artemia salina*) was investigated using anticancer agent vincristine sulphate as reference standard (Meyer *et al.*, 1982). In a single day experiment, a range of concentrations of sample solutions in dimethylsulfoxide (DMSO) was used and nauplii were counted *via* visual inspection to measure their mortality.

**Membrane stabilizing assay:** The membrane stabilizing activity of *B. clarkei* leaves was evaluated by measuring the inhibition of hemolysis of human erythrocyte in hypotonic solution- and heat-induced condition and anti-inflammatory agent acetyl salicylic acid was considered as standard (Shinde *et al.*, 1999). For calculation given equations were followed.

%Inhibition of hypotonic solution induced hemolysis

$$= \left( 1 - \frac{\text{O. D. of test sample in hypotonic solution}}{\text{O. D. of hypotonic buffered saline solution alone}} \right) \times 100$$

%Inhibition of heat induced hemolysis

$$= \left[ 1 - \left( \frac{\text{O. D. of heated test sample} - \text{O. D. of unheated test sample}}{\text{O. D. of heated control sample} - \text{O. D. of unheated test sample}} \right) \right] \times 100$$

**Thrombolytic activity:** Following previously devised method (Prasad *et al.*, 2006), thrombolytic activity of the samples was expressed by evaluating the clot lysis of blood drawn from human volunteers using streptokinase as standard reference. The

experiment was performed following ethical guidelines described in the declaration of Helsinki 2013 under the approval of the institutional ethical review committee and consents from all the human

volunteers were taken. The given equation was followed to calculate clot lysis.

$$\% \text{ Clot lysis} = \left( \frac{\text{Weight of the clot after lysis}}{\text{Weight of clot before lysis}} \right) \times 100$$

**Central analgesic activity:** Central analgesic activity was screened as per the established protocol of Pizziketti *et al.* (1985) where the negative control group was given 1% Tween 80 in saline mixture (0.1 mL/10mg) orally and morphine was utilized as reference standard. Heat stress was used as a pain stimulus that was applied to the mouse's tail. After the introduction of plant materials (BC 200, BC 400), tail immersion time was recorded at 30, 60, and 90 minutes. The percent of pain inhibition was calculated using the following formula:

$$\% \text{ Inhibition of writhing} = \frac{\text{Mean no. of writhing (control)} - \text{Mean no. of writhing (test)}}{\text{Mean no. of writhing (control)}} \times 100$$

**Hypoglycemic activity:** The tail tipping method was used to determine the hypoglycemic action of the extracts (Durschlag *et al.*, 1996). The control group was administered with 1% Tween 80 in normal saline while glibenclamide was given as the standard. Before application of any kind of extract or drug, the blood glucose level of each animal was measured by a glucometer (Bioland G-423 S). 10% glucose solution (2 gm/kg b.w.) was applied to each experimental animal of every group after 30 mins of administration of extract or drug. After that, blood was withdrawn from the vein of mouse's tail at 60,

$$\% \text{ Inhibition of defecation} = \frac{\text{Mean defecation of control} - \text{Mean defecation of test sample or standard}}{\text{Mean defecation of control}} \times 100\%$$

**Statistical analysis:** For all bioassays, the data are presented as mean  $\pm$  standard error of mean (M $\pm$ SEM) of three replicates of each sample. Student's t-test was used to affirm the significance between the control and experimental group of animals where the p values < 0.05 were considered to be statistically significant.

$$\text{Pain inhibition percentage (PIP)} = \left[ \frac{T_1 - T_0}{T_0} \right] \times 100$$

where,  $T_1$  = Post-drug latency,  $T_0$  = Pre-drug latency

**Peripheral analgesic activity:** Acetic-acid induced writhing method narrated by Koster *et al.* (1959) was implemented to ascertain the peripheral analgesic action where intra-peritoneal acetic acid (0.1mL) acted as the source of pain sensation. The control group was given 1% Tween 80 in saline and the standard group received diclofenac sodium (5mg/kg b.w.) while the test groups were administered with two different doses (BC 200, BC 400). By applying the following formula, the percent inhibition of writhing was calculated.

120, and 180 mins and the plasma glucose level was measured to observe hypoglycemic action.

**Antidiarrheal activity:** Castor oil-induced diarrhea method stated by Shoba and Thomas (2001) was performed to evaluate antidiarrheal action of the leaves extract. Negative control group was fed by 1% Tween-80 in water, while oral loperamide at 5 mg/kg was utilized as reference standard. The crude extract was given orally to the mice residing in test groups. The number of fecal stool spots was documented for each mouse. The inhibition of defecation in mice was reckoned in percentage by using the following equation.

## Results and Discussion

Bioactivities of *B. clarkei* leaves were evaluated following both *in vitro* (antioxidant, cytotoxic, membrane stabilizing, thrombolytic) and *in vivo* (antidiarrheal, analgesic, hypoglycemic) assays in Swiss mice model.

**Total phenolic content:** The total phenolic content of the extractives was found to be in the range of 17.62 -71.59 mg of GAE/gm of extractives (Table 1). Though, both ethyl acetate (EASF) and aqueous soluble extractives (AQSF) showed a substantial amount of phenolic content the highest is expressed by ethyl acetate soluble fraction.

**DPPH free radical scavenging activity:** Strikingly, the EASF also displayed the maximum DPPH free radical scavenging activity among all the samples ( $IC_{50}$  value 19.25  $\mu\text{g/ml}$ ) as compared to standard BHT and AA which showed  $IC_{50}$  value of 11.76 and 3.08  $\mu\text{g/ml}$ , respectively (Table 1). This result further justifies the previously studied correlation between phenolic phytoconstituents and free radical neutralizing properties; antioxidant potential of a plant in general (Casquete *et al.*, 2015). As flavonoids and diterpenes isolated from plant source be related to their antioxidant potential (Alam *et al.*, 2021; Deghima *et al.*, 2021) along with existing reports on the isolation of said compounds from other plants of the genus (Chen *et al.*, 2009), it can be fairly speculated that flavonoids or diterpenes present in *B. Clarkei* leaves might be responsible for its antioxidant activity.

**Brine shrimp lethality assay:** The aqueous soluble fraction (AQSF) showed the highest cytotoxicity effect with  $LC_{50}$  value of 18.60  $\mu\text{g/ml}$  as compared to standard reference VS (0.45  $\mu\text{g/ml}$ ). The crude methanol extract expressed almost similar cytotoxicity (18.65  $\mu\text{g/ml}$ ) which proves the probable presence of cytotoxic constituents in the relatively polar fractions of *B. clarkei* leaves (Table 1).

**Membrane stabilizing activity:** The membrane stabilizing activity of the extractives was also evaluated by measuring the inhibition of hypotonic and heat-induced hemolysis of human erythrocytes. The DCM soluble fraction (DCMSF) revealed the maximum inhibition of hemolysis in hypotonic solution induced condition (30.64%) while aqueous soluble extractive (AQSF) inhibited heat-induced RBC hemolysis by 39.15% as compared to 61.98% and 41.12% inhibition displayed by ASA in respective conditions (Table 1). Due to the

resemblance between erythrocyte membrane and lysosomal membrane, the effect of an agent on erythrocyte stabilization could be extrapolated to assume the effect on lysosomal membrane which further correlates with the agent's anti-inflammatory property (Omale and Okafor, 2008). It is well known lysosomes actively contribute to the release of inflammatory mediators such as histamine, prostaglandin, leukotriene etc. (Shinde *et al.*, 1999) and produce free radicals damaging cellular macromolecules which augment the state of inflammation (Valko *et al.*, 2007). Thus, constituents responsible for membrane stabilizing activity of *B. clarkei* extract might provide opportunities to develop drug candidates to treat inflammatory diseases.

**Thrombolytic activity:** The crude methanol extract of the *B. clarkei* leaves along with its organic and aqueous fractions were assayed for thrombolytic potential by determining their ability to lysis blood clot. The samples exhibited thrombolysis in the range of 17.45-30.47% as compared to 72.24% clot lysis for standard streptokinase (Table 1). The crude extract (MESF) revealed the highest clot lysis ability which is suggestive of its therapeutic potential in different thromboembolic diseases.

**Central analgesic activity:** The percent tail immersion time elongation revealed by dose 200-mg/kg body weight (BC 200) and 400-mg/body weight (BC 400) after 90 mins of administration were respectively 262.07% and 310.70%, (Table 2) which suggested the augmentation of flicking response in a manner which was dose and time dependent. Heat-induced pain sensation is believed to follow various intricate ways which include the central system of dopamine, opiate, serotonin, and descending noradrenergic neurons (Mishra *et al.*, 2011). The central analgesic effect of both doses of the crude extract showed a promising increase in pain threshold which was possibly due to the interference with the central mechanism. It may be involved with these receptor systems or the inhibition of different kinds of endogenous substances which play a key role in pain stimulation.

**Table 1. Total phenolic content, DPPH radical scavenging, cytotoxicity, membrane stabilizing and thrombolytic activities of *B. clarkei* leaves.**

| Sample/<br>standard | Total phenolic<br>content<br>(mg of GAE/gm<br>of extract) | DPPH Free<br>radical scavenging<br>activity<br>(IC <sub>50</sub> µg/ml) | Cytotoxicity<br>(LC <sub>50</sub> µg/ml) | Membrane stabilizing activity (%) |                          | Thrombolytic<br>(% clot lysis) |
|---------------------|---|---|--|-----------------------------------|--------------------------|--------------------------------|
|                     |   |   |  | Hypotonic<br>solution induced     | Heat solution<br>induced |                                |
| MESF                | 17.62±0.35  | 77.59±0.31  | 18.65±0.23                               | 9.72±0.20                         | 25.27±0.29               | 30.47±0.23                     |
| PESF                | 22.07±0.30  | 54.18±0.18  | 19.73±0.23                               | 16.14±0.11                        | 34.93±0.34               | 28.52±0.26                     |
| DCMSF               | 33.63±0.35  | 83.46±0.12  | 80.84±0.22                               | 30.64±0.33                        | 36.26±0.29               | 23.52±0.29                     |
| EASF                | 71.59±0.32  | 19.25±0.62  | 33.52±0.16                               | 26.25±0.29                        | 38.74±0.32               | 17.45±0.35                     |
| AQSF                | 64.25±0.16  | 44.96±0.22  | 18.60±0.17                               | 22.75±0.30                        | 39.15±0.09               | 20.36±0.34                     |
| BHT                 |   | 11.76±0.34  |  |                                   |                          |                                |
| AA                  |   | 3.08±0.09   |  |                                   |                          |                                |
| VS                  |   |   | 0.45±0.01                                |                                   |                          |                                |
| ASA                 |   |   |  | 61.98±0.13                        | 42.12±0.35               |                                |
| SK                  |   |   |  |                                   |                          | 72.24±0.41                     |

Values are expressed as Mean ± SEM (n=3). MESF= Methanol crude extract; PESF= Pet-ether soluble fraction; DCMSF= DCM soluble fraction; EASF= Ethyl acetate soluble fraction; AQSF= Aqueous soluble fraction; BHT= Butylated hydroxyl toluene; AA= Ascorbic acid, VS= Vincristine sulfate, ASA= Acetyl acetic acid, SK= Streptokinase

**Table 2. Central analgesic activity of crude methanol extract of *B. clarkei* leaves.**

| Animal<br>groups | After 30 mins  |              | After 60 mins |              | After 90 mins |              |
|------------------|----------------|--------------|---------------|--------------|---------------|--------------|
|                  | M±SEM          | % Elongation | M±SEM         | % Elongation | M±SEM         | % Elongation |
| CTL              | 2.11 ± 0.12    | -            | 2.24±0.04     | -            | 1.98±0.19     | -            |
| STD              | 5.46 ± 0.07*** | 158.20       | 9.15±0.07***  | 308.99       | 13.38±0.15*** | 574.57       |
| BC 200           | 3.61 ± 0.12*** | 70.77        | 5.63±0.14***  | 151.79       | 7.18±0.12***  | 262.07       |
| BC 400           | 4.35 ± 0.11*** | 105.84       | 6.03±0.13***  | 169.27       | 8.15±0.28***  | 310.70       |

Values are expressed as Mean ± SEM (n=3). \*\*\*p<0.001, \*\*p< 0.01, \*p< 0.05 compared to control compared to negative control. CTL = negative control (1% Tween 80 in water), STD = positive control (Morphine at 2mg/kg b.w.), BC 200 = Methanol extract of *B. clarkei* whole plant at 200 mg/kg b.w., BC 400 = Methanol extract of *B. clarkei* whole plant at 400 mg/kg b.w.

*Peripheral analgesic activity:* The crude extract exhibited significant peripheral analgesia at both doses (BC 200, BC 400) respectively suggested by 47.54% and 65.57% reduction of acetic acid initiated writhing, while standard diclofenac sodium showed 85.25% of inhibition (Table 3). As previously reported, the acetic acid-induced pain might be due to the involvement of abdominal constriction and the activation of the local peritoneal chemosensitive nociceptors (Bentley *et al.*, 1983; Sani *et al.*, 2013; Schumacher *et al.*, 1940). Therefore, it can be

deduced that the plant extract may have intervened with these peritoneal receptors to exhibit peripheral analgesic activity. Alongside, the association of increased levels of prostaglandins such as PG-E2 and PG-F2α (Deradtet *et al.*, 1980) and lipooxygenases have been found in this irritant principle of pain induction. So it can be stipulated that the underlying mechanism of analgesia may be linked to the inhibition of certain endogenous substances' release such as cyclooxygenases and/or lipooxygenases (Sani *et al.*, 2013).

**Hypoglycemic activity:** Result of the study suggested significant blood glucose reduction by the plant extract whereas the percent decrease of plasma glucose level was found to be dose and time-dependent. The highest hypoglycemic action of 64% was exhibited by the dose of 400-mg/kg after 180 minutes (Tables 4, 5). Numerous studies stated that hypoglycemic activity may be due to the regulation of insulin secretion from  $\beta$  cell of islets of Langerhans or increased sensitization of insulin

receptor towards insulin which eventually result in blood glucose level reduction (Khan *et al.*, 2010). Other mechanisms may include inhibition of glucose absorption from the intestine, increased activity of gluconeogenic enzymes, elevated muscle uptake of blood glucose, etc. (Joseph *et al.*, 2013). As this study represented remarkable hypoglycemic action of test extracts compared to standard, *B. clarkei* extracts can be considered as a potential source of hypoglycemic agents.

**Table 3. Peripheral analgesic activity of crude methanol extract of *B. clarkei* leaves.**

| Animal group | Writhing Count |     |     | Number of writhing (Mean $\pm$ SEM) | Inhibition (%) |
|--------------|----------------|-----|-----|-------------------------------------|----------------|
|              | M-1            | M-2 | M-3 |                                     |                |
| CTL          | 20             | 22  | 19  | 20.33 $\pm$ 0.88                    | -              |
| STD          | 3              | 2   | 4   | 3.00 $\pm$ 0.58***                  | 85.25          |
| BC 200       | 10             | 12  | 10  | 10.67 $\pm$ 0.67***                 | 47.54          |
| BC 400       | 7              | 6   | 8   | 7.00 $\pm$ 0.58***                  | 65.57          |

Values are expressed as Mean  $\pm$  SEM (n=3). \*\*\*p<0.001, \*\*p< 0.01, \*p< 0.05 compared to control compared to negative control. CTL = negative control (1% Tween 80 in water), STD = positive control (Diclofenac sodium at 5mg/kg body weight). M-1, M-2, M-3 = Mice 1, Mice 2, Mice 3 respectively.

**Table 4. Plasma glucose level (mmol/L) of mice in hypoglycemic activity test.**

| Animal group | 30 min (mmol/l)     | 60 min (mmol/l)   | 120 min (mmol/l)   | 180 min (mmol/l)   |
|--------------|---------------------|-------------------|--------------------|--------------------|
| CTL          | 26.37 $\pm$ 0.44    | 13.13 $\pm$ 0.70  | 10.63 $\pm$ 0.37   | 7.97 $\pm$ 0.40    |
| STD          | 22.07 $\pm$ 1.78    | 7.27 $\pm$ 1.06** | 3.97 $\pm$ 0.48*** | 1.87 $\pm$ 0.47*** |
| BC 200       | 17.40 $\pm$ 2.36*   | 11.03 $\pm$ 0.33  | 8.17 $\pm$ 0.75*   | 5.00 $\pm$ 0.87**  |
| BC 400       | 13.80 $\pm$ 0.93*** | 8.53 $\pm$ 1.42*  | 7.30 $\pm$ 1.40    | 2.90 $\pm$ 1.08**  |

Values are expressed as Mean  $\pm$  SEM (n=3). CTL = negative control (1% Tween 80 in water), STD = positive control (Glibenclamide at 5mg/kg b.w.). \*\*\*p<0.001, \*\*p< 0.01, \*p< 0.05 compared to control compared to negative control.

**Table 5. Hypoglycemic activity of methanol extract of *B. clarkei* leaves.**

| Animal groups | Dose           | % Reduction of Blood Glucose |           |            |            |
|---------------|----------------|------------------------------|-----------|------------|------------|
|               |                | 25 minute                    | 60 minute | 120 minute | 180 minute |
| CTL           | -              | -                            | -         | -          | -          |
| STD           | 5 mg/kg b.w.   | 16%                          | 45%       | 63%        | 77%        |
| BC 200        | 200 mg/kg b.w. | 34%                          | 16%       | 23%        | 37%        |
| BC 400        | 400 mg/kg b.w. | 48%                          | 35%       | 31%        | 64%        |

CTL = negative control (1% Tween 80 in water), STD = positive control (Glibenclamide).

**Table 6. Antidiarrheal activity of methanol extract of leaf of *B. clarkei*.**

| Animal group | Dose           | Number of diarrheal feces (Mean $\pm$ SEM) | % Reduction of diarrhea |
|--------------|----------------|--|-------------------------|
| CTL          | 10 ml/kg b.w.  | 8.67 $\pm$ 0.58                            | -                       |
| STD          | 5 mg/kg b.w.   | 2.33 $\pm$ 0.58***                         | 73.08                   |
| BC 200       | 200 mg/kg b.w. | 4.00 $\pm$ 10***                           | 53.85                   |
| BC 400       | 400 mg/kg b.w. | 2.67 $\pm$ 0.58**                          | 69.23                   |

Values are expressed as Mean  $\pm$  SEM (n=3). CTL = negative control (1% Tween 80 in water), STD = Positive control (Loperamide at 5mg/kg body weight). \*\*\*p<0.001, \*\*p< 0.01, \*p< 0.05 compared to control compared to negative control

**Antidiarrheal activity:** In comparison with the control group, *B. clarkei* leaves (BC 200, BC 400) displayed significant dose-dependent reduction of feces (53.85% and 69.23%), respectively (Table 6). The active constituent of castor oil, ricinoleic acid is known to stimulate peristalsis in the small intestine which changes the water, electrolyte permeability of the intestinal wall and causes inflammation of the mucosa of the intestine due to prostaglandins secretion; augmenting diarrhea (Maniyar *et al.*, 2010). Moreover, flavonoids, diterpenes, sesquiterpenes, and terpenoid derivatives are linked to autacoids and prostaglandins release inhibition, thereby antagonizing the motility and secretion caused by castor oil (Milanova *et al.*, 1995; Nikiema *et al.*, 2001). Previous reports on the isolation of those mentioned compounds from other plants of the genus may justify the aforementioned mechanism of antidiarrheal activity (Chen *et al.*, 2009; Guan *et al.*, 2012; Jahan *et al.*, 2014).

### Conclusion

It is evident from the above findings that the relatively polar extractives of leaves of *B. clarkei* possess prominent antioxidant, cytotoxic, membrane stabilizing properties, and thrombolytic potentials. In mouse model, the plant also displayed significant dose and time-dependent analgesic (central, peripheral), hypoglycemic and antidiarrheal properties. These findings justify the abundant ethnopharmacological uses of this genus. This warrants further thorough phytochemical investigations to isolate constituents responsible for the reported biological activities.

### Declaration

All authors read the manuscript and approve it for the publication and no part of this manuscript has been published before in any journal.

### Conflict of interest

The authors state that they have no conflict of interest.

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This work has not received any fund.

### Availability of data and materials

All the findings have been incorporated in this manuscript.

### Authors' contribution

MAS and TJ conceptualized the work. RAR and FK collected the plant materials and conducted the lab works. FK and AUJ wrote the manuscript. AUJ, FK and TS analyzed the data. SA and MAI critically evaluated the manuscript. MAS and SA edited and drafted the final manuscript.

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