Kathmandu University
Journal of Science, Engineering and Technology

Gauchan *et. al.*, Vol. 12, No. II, December, 2016, pp 88-95.

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

BAMBUSA BALCOOA ROXB. : A NOVEL REMEDY FOR PEPTIC ULCER

Anil Upreti, Bibek Byanju, Binod Pokhrel, Samir Kuinkel, Arjun Bhusal, Nita Thapa, Meena M. Suwal, Janardan Lamichhane, Dhurva P. Gauchan*
Department of Biotechnology, Kathmandu University, Dhulikhel, Kavre, Nepal

*Corresponding author's email: gauchan@ku.edu.np
Received 16 December, 2016; Revised 27 December, 2016

ABSTRACT
Analysis of bamboo species namely *B. balcooa* obtained from the Sindhuli district of Nepal was carried out. Phytochemical analysis between the stem and leaves extract in six solvent was carried out. Highest yield of 34.55% of *B. balcooa* leaves in methanol as extracting solvent was obtained. Phytochemical analysis exhibited the presence of sterols, coumarins, reducing sugar, cardiac glycoside, flavonoids, and other polyphenolic compounds. Pharmacological analysis of methanol *B. balcooa* leaves extract revealed anti- ulcer activity of (14.66% protective ratio). The data are expressed as Mean ±SD which were further subject statistical analysis using Origin Pro and SPSS.

INTRODUCTION
Bamboos are perennial plants belonging to grass family Poaceae with scattered vascular bundle in cylindrical arrangement [1]. *Bambusa balcooa,* a female bamboo is tropical clumping bamboo, culms can be up to 30 meters but in general are less than 18 meters [2]. Multiple researchers have reported the use of the *B. balcooa* in construction purpose acknowledging the mechanical strength and agricultural implementation. The values of *B. balcooa* are generally limited in construction materials, paper industries [3].

The hidden treasure of the arsenal of compound possessed by the green biomass along with usefulness in various aspect such as pharmacological aspect has been hidden in light of the researching dominantly focusing in the micro propagation and similar studies. The result suggested by multiple authors suggesting compounds like Phenols, terpenoids, flavonoid tannins play curative and good effect in health has been studied.[4] The presence of flavonoids, tannins in *B. balcooa* has significant anti-ulcer activity maintaining the cellular integrity of gastric mucosa. The anti-oxidant activity possessed by *B. balcooa* extract strengthens the mucosal defense system through gastric mucus secretion [5]. To our report this is the first analysis in Nepal focusing in the *B. balcooa* for anti-ulcer activity.

MATERIALS AND METHODS

Sample Collection
The plants were collected from Janakpur Zone and Sindhuli district, 27.2569° N, 85.9713° E. The leaves were air dried in the shade at the room temperature, were protected from direct sunlight whereas other group was placed in direct sunlight. Plants were collected in the polythene bags during morning hours and placed in the ice box for preventing any contamination and preservation was done.

Gauchan *et. al.,* Vol. 12, No. II, December, 2016, pp 88-95.

Plant Extraction

The shade dried and sun light dried leaves were weighed 50gm and extraction was done using orbital shaker at the continuous rate of 80 rpm with six solvents for 24 hrs., intended for efficient extraction. The solvent was filtered through the ash less whatman no.1 filter paper and the extract was concentrated further and stored for further analysis.

Phytochemical Screening

The phytochemical analysis of Alkaloids, Flavonoids, Phenolic content, Saponin, Sterols, Cardiac glycoside, Carbohydrate, Protein, Starch, Volatile oils, Tannin, Terpenoid, Glycoside, Phytosterols, Diterpenes, Flavonoid and Reducing compound was performed following the standard protocol with slight modification [6, 7, and 8].

Mice Model

Healthy albino mice of 20-50gm of either sex were used for present study. The animals were housed in polypropylene cages maintained under standard condition (12 hour light and 12 hour dark cycle, 25±5°C and 40-60% humidity). They were fed with standard mice pellet diet and free access to water was provided. All the animal experiments were conducted according to the ethical norms approved by, Ethical committee Department of Plant Resources.

Anti-Ulcer

Different groups of mice were used to study the effects of Ethanol and Methanol. The mice were divided into Four groups each consisting of six mice. The group I: normal/control animals received D/W, per body weight orally, group-II: ulcer inducer per body weight, rhinitidine was used as standard, group-III: ulcer inducer per body weight with ethanol extract and group-IV: ulcer inducer per body weight with methanol extract. The percentage protection index was calculated following the standard protocols [9].

RESULTS AND DISCUSSION

Physical Characters and Phytochemical comparison between sun dried and shed dried

The extract was subjected to the extraction and the color, aroma and yield was determined. The highest yield in the leaf extract was determined to be of methanol with the 34.55% and the least extract was observed to be 1.77% stem hexane extract. The extract obtained from the cold extraction of the sun and shed dried sample were subject to the phytochemical analysis for the determination of the distinct difference between the sun and shed dried extract. No distinct difference was obtained even in the phytochemical analysis showing no major differences. The methanol , 50% Ethanol and water extract showed the better result of the presence of phytochemical compared to the other solvents concluding the fact of presence of polar phyto-chemical then non polar. The major phytochemical detected were found to be Saponin, Phenols, Flavonoid, Terpenoid, Sterols, Coumarin, Reducing sugar and Cardiac Glycoside (Table 1).

Kathmandu University
Journal of Science, Engineering and Technology

Gauchan *et. al.*, Vol. 12, No. II, December, 2016, pp 88-95.

Table 1. Physical Characters of the Extract

Solvents	Plant Part	% Yield	Color	Aroma
Hexane	Stem	1.77	Pale White	Herbal
Acetone		3.08	Light Green	Herbal
Ethyl Acetate		1.18	Light Green	Herbal
50% Ethanol		16.18	Dark Brown	Slight Herbal
Methanol		9.62	Yellow	Herbal
Water		6.20	Light Brown	Rotten Like
Hexane	Leaves	2.04	Pale White	Herbal
Acetone		5.11	Dark Green	Herbal
Ethyl Acetate		2.27	Light Green	Herbal
50% Ethanol		12.8	Turbid Brown	Slight Herbal
Methanol		25.67	Dark Brown	Herbal
Water		10.58	Dark Brown	Rotten Like

Out of the six solvents used methanol, aq. Ethanol and water proved to be the best solvents for the extraction (Table 2-4). The non-polar solvent Hexane proved to be ineffective for the extraction as no phytochemical presence was detected. Similar was the condition of for acetone and ethyl acetate. Significant phytonutrients and phytochemicals were present in the more polar and semi polar solvents i.e. water, aq. ethanol and methanol. This indicates the highly presence of the polar components in the *B. balcooa* extract. The enriched phytochemicals in the polar and semi polar solvent narrowed our research just to two solvents methanol and aq. Ethanol hence after.

Table 2. Dried Sample of *B. balcooa* Phytochemical Analysis

Test	Hexane		Acetone	
	Leaves	Stem	Leaves	Stem
Saponin	+	+	++	-
Phenol	-	-	-	-
Quinones	-	-	+	++
Terpenoids	-	-	-	-
Protein	-	-	-	-
Sterols	-	-	-	-
Flavonoids	-	-	-	-
Flavones	-	-	-	-
Reducing Sugar	-	-	-	-
Alkaloid	-	-	-	-
Resins	-	-	-	-
Starch	-	-	-	-
Carbohydrate	-	-	-	-
Ninhydrin	-	-	-	-
Tanin	+	+	+	+

Kathmandu University
Journal of Science, Engineering and Technology

Table 3. Dried Sample of *B. balcooa* Phytochemical Analysis

Test	Ethyl Acetate		Methanol	
	Leaves	Stem	Leaves	Stem
Saponin	+	-	++	+
Phenol	-	-	++	-
Quinones	+	-	++	++
Terpenoids	+	-	++	+
Protein	-	-	+	-
Sterols	-	-	++	+
Flavonoids	-	-	++	-
Flavones	-	-	-	+
Reducing Sugar	-	-	+	+
Alkaloid	-	-	-	-
Resins	-	+	+	+
Starch	-	-	-	-
Carbohydrate	-	-	-	+
Ninhydrin	-	-	++	-
Tanin	+	+	+	+

Table 4. Dried Sample of *B. balcooa* Phytochemical Analysis

Test	50% Ethanol		Water	
	Leaves	Stem	Leaves	Stem
Saponin	++	++	+	++
Phenol	+	-	++	-
Quinones	+	+	++	+
Terpenoids	+	++	++	+
Protein	+	+	+	+
Sterols	++	+	-	-
Flavonoids	++	-	-	-
Flavones	-	-	++	+
Reducing Sugar	+	-	+++	+
Alkaloid	-	-	-	-
Resins	+	+	+	+
Starch	-	-	-	-
Carbohydrate	-	-	-	+
Ninhydrin	++	-	++	-
Tanin	++	+	+	+

Kathmandu University
Journal of Science, Engineering and Technology

Anti-Ulcer Activity

The *B. balcooa* leaf has shown satisfactory protective ratio of 14.44% compared to that of Standard ranitidine with protective ratio of 60%.The pH of stomach was found to be slightly acidic i.e., 5.41 in case of standard whereas the induced peptic ulcer showed the prominent pH of 3.58. The result was found to be significant at $p<0.05$.

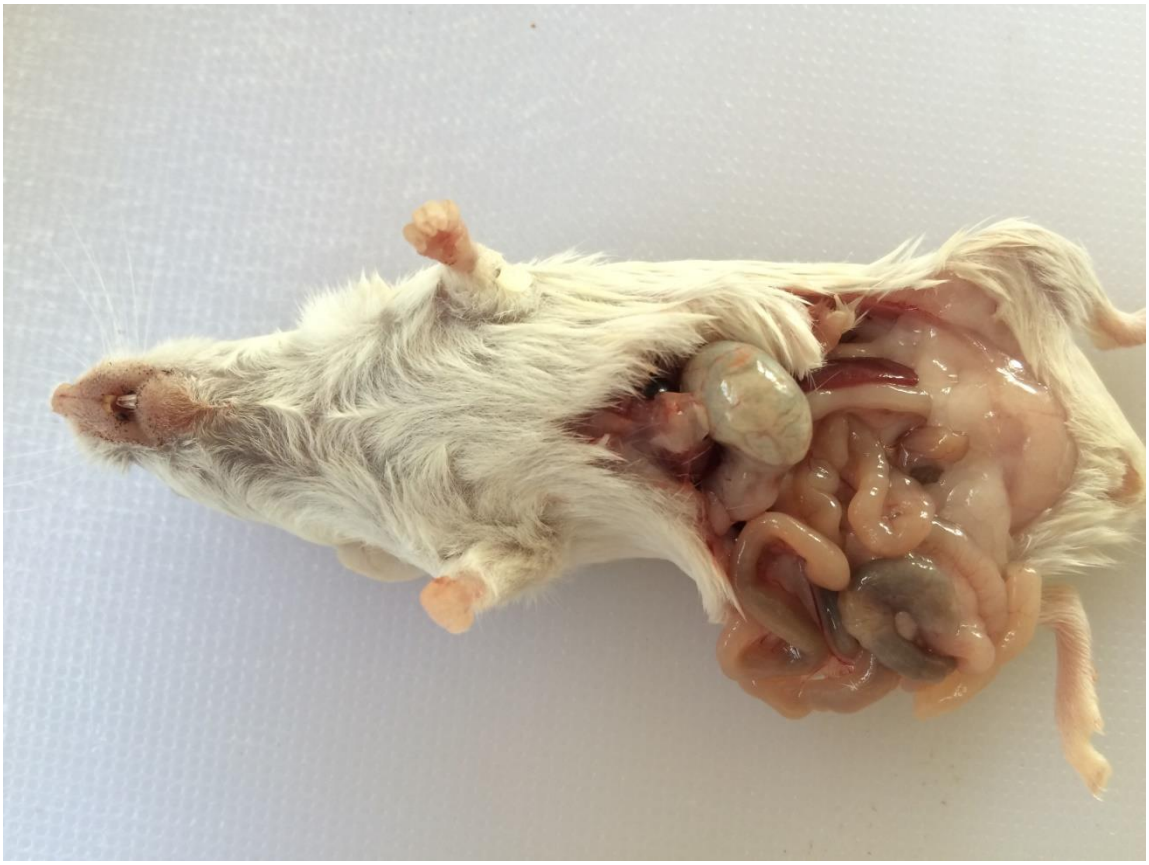

Figure 1. Dissection of Mice after Ulcer Induction

Independent of gastric acid secretion ethanol directly damages the mucosa resulting in the gastric ulceration [10]. Study has shown that the result may be due to secretary product of mast cell [8] and reactive oxygen species [11, 12]. In the ethanol-induced ulcer model, Administration of ethanol resulted in significant ulcers in the control group, with significant reduction in the treated groups. Anti-Ulcer activity was evaluated by considering the parameters commonly known as ulcer index which are the factors such as reduction in the gastric volume, decrease in free and total acidity. The disturbance of defensive factors like mucosal blood flow and mucus secretion has been reported to cause ulcers [13]. So the protective effect of methanol extract of *B. balcooa* may be due to protection against 5-lipooxygenase or leukotriene pathway or possible stimulation of prostaglandin synthesis which in turn is involved in cytoprotection of gastric mucosa. [14, 15]. The various phytochemicals involved may have direct effect in the protective activity.

Kathmandu University
Journal of Science, Engineering and Technology

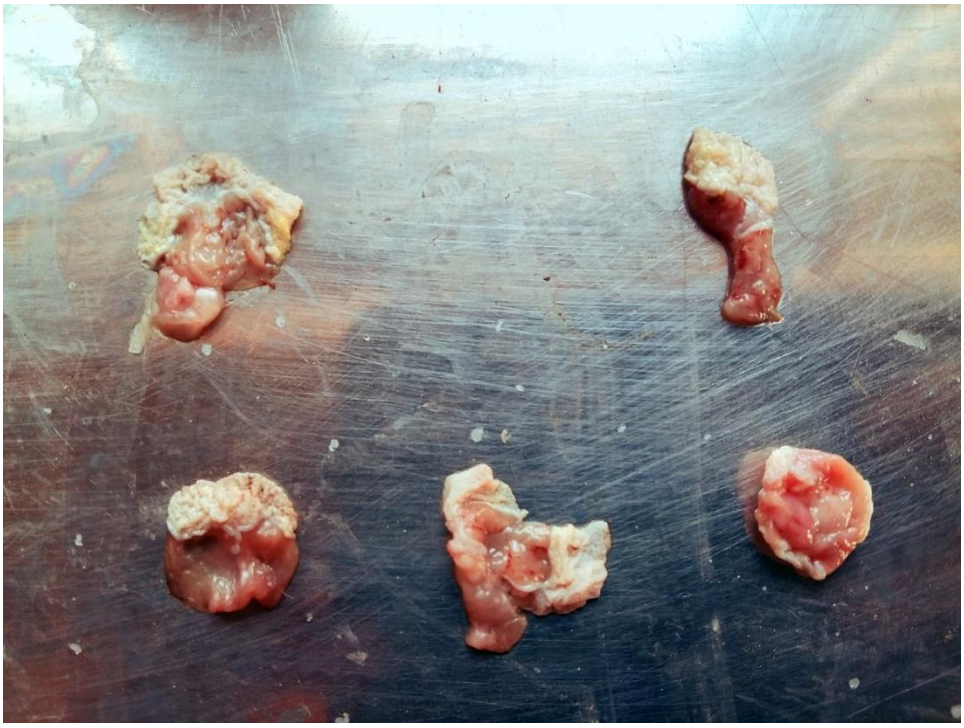

Figure 2. Comparison of Mice Stomach for Cure of Peptic Ulcer

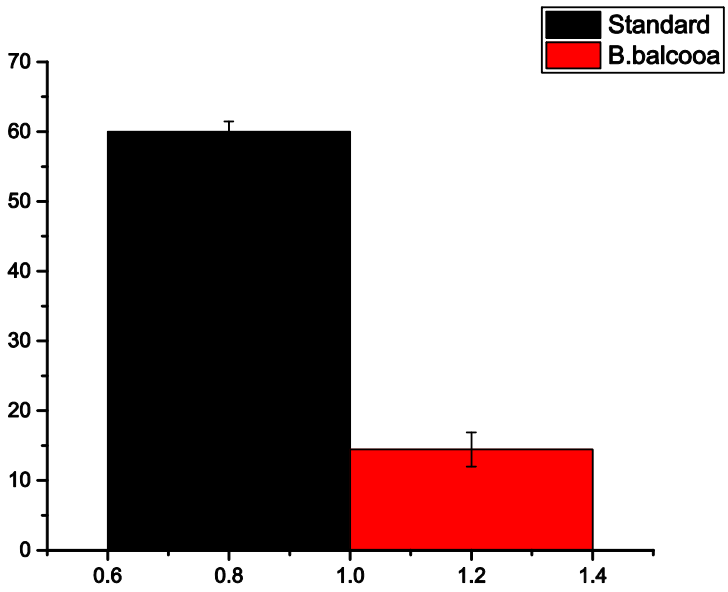

Figure 3. Anti- Ulcer Activity of B. *balcooa* represented as Mean±SD

Gauchan *et. al.,* Vol. 12, No. II, December, 2016, pp 88-95.

The detailed analysis further followed by the fraction purification, compound identification with the analysis in animal model can identify the actual responsible compound involved in the cure and further the cytotoxicity can be analyzed which may act as a precursor for novel drug discovery. The bamboo is arsenal of the phytochemical and other secondary metabolite. Taking in regards its wide availability and easy accessibility can mitigate the multiple problems recently faced in this green biosphere. Further the research has provided the scientific basis for the development and use of noble anti-ulcer drugs derived from the *B. balcooa* extract.

ACKNOWLEDGEMENT

We are privileged to articulate our cordial thanks to President Chure Terai-Madesh Conservation Project for supporting us with financial assistance in our research along with Natural Product Research Laboratory (NPRL), Ministry Of Forest and Soil Conservation in Department of Plant Resources (NPRL) for providing us the mouse models and Kathmandu University.

We acknowledge and show appreciation to Research Assistant Ms. Sudina Bhuju, Mr. Santosh Dhamala, Mrs. Bishnu KC and Mr. Saugat Shrestha, who have facilitated us to complete this project. We extend our gratitude to all who have deliberately or unknowingly added a brick in the completion of this project.

REFERENCES

[1] Botany; Wilson,C L & Loomis W E, Third edition, Holt, Rinehart and Winston.

[2] "Bambusa Balcooa - Useful Tropical Plants", *Tropical Theferns Info*, 2016, http://tropical.theferns.info/viewtropical.php?id=Bambusa+balcooa

[3] Ramanuja Rao, I. V et al. *Bamboos*. 1st ed., Peechi, Kerala Forest Research Institute, India, 1990

[4] Anil S S, Preliminary Phytochemical Analysis And Antimicrobial Potential Of Fermented Bambusa Balcooa Shoots, *An International Quarterly Journal Of Life Science*, 2012, 1.

[5] Onasanwo & Samuel Adetunji *et. al*., Anti-ulcer and ulcer healing potentials of Musa Sapientum Peel extract in the laboratory rodents, *Pharmacognosy Research*, 5 (2013) 3, 173. *Medknow*, doi:10.4103/0974-8490.112423.

[6] Ciulei I, Practical Manuals on the Industrial Utilization of Chemical and Aromatic Plants, *Methodology for Analysis of Vegetable Drugs*, 1st Edn., Ministry of Chemical Industry, Bucharest, 1982, 67.

[7] Yadav R N S & Munin A, Phytochemical analysis of some medicinal plants, *Journal of Phytology*, 3(2011) 12, 10-14

[8] Prashant T, Bimlesh K, Mandeep K, Gurpreet K & Harleen K, Phytochemical screening and Extraction: A Review, *Internationale Pharmaceutica Sciencia*, 2011.

[9] Noemi D P, Darryl H B C, Christine L Chichioco-Hernandez, Anti-ulcer activity of Leguminosae plants, *Arq. Gastroenterol*, 51 (2014) 1.

[10] Takase H, Yamamoto K, Hirano H, Saito Y & Yamashita A, Pharmacological profile of gastric mucosal protection by marmin and nobiletin from a traditional herbal medicine, Aurantii Fructus Immaturus, *Jpn J Pharmacol*, 66 (1994), 139-147.

[11] Pihan G, Regillo C & Szabo S, Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury, *Dig Dis Sci.*, 32 (1987), 1395-1401

[12] Mizui T, Sato H, Hirose F & Doteuchi M, Effect of anti-peroxidative drugs on gastric damage induced by ethanol in rats, *Life Sciences*, 41 (1987), 755-7637

[13] Goel R K & Bhattacharya S K, Gastro duodenal mucosal defense and mucosal protective agents, *Indian J. Exp. Biol.*, 29 (1991), 701-14

[14] Rang H P, Dale M M, Ritter J M & More P K, Local hormones, inflammation and immune reaction, *In: Pharmacology*, 5th ed., Elsevier Science, Churchill Livingstone, Bath Press, UK, 2003, 231-36.

[15] Hardman J G, Limbird L E & Goodman Gilman A, Lipid Derived Autacoids, *The Pharmacological Basis of Therapeutics*, 9th ed., Mc Graw-hill, NewYork, 1996, 601-06.