Chapter - 7. SUMMARY, CONCLUSION & RECOMMENDATIONS
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Summary:

To present succinctly, it can be stated that the present investigation, pharmacognostic, phytochemical and pharmacological properties were carried out to ascertain the ethnobotanical claims of pharmacological potentials of the leaves of *Basella rubra* and *Phyllanthus acidus*.

The leaves of *Phyllanthus acidus* and *Basella rubra* were collected and authenticated by its morphological and histological characters. The percentage of macronutrients like sodium, potassium, and magnesium were within the stipulated limits. Toxic and heavy metals resembling palladium, arsenic, lead and mercury were within the limit and ensure the safety of the study.

Preliminary phytochemical studies established the presence of tannins, steroids, triterpenoids, carbohydrates, flavonoids and proteins in EEBR as well as steroids, triterpenoids, flavonoids, tannins, triterpenoids, phenolics, carbohydrates and proteins in EEPA.

The results of the acute toxicity studies of EEPA and EEBR as per OECD guidelines 425 did not show any sign and symptoms of toxicity or mortality upto 2000 mg/kg, hence considered as category 5.

Results of behavioural nociceptive tests of the EEBR and EEPA conducted by using five different methods based on acute and sub-acute,
which differ with respect to stimulus eminence, intensity and extent, were employed in evaluating the analgesic effect to ascertain the analgesic properties of EEBR as well as EEPA showed appreciable and dose related efficacy in all the administered doses which may be mediated via central and peripheral pain inhibitory mechanisms.

Results of anti-inflammatory studies by four different models namely carrageenan, histamine, dextran and cotton pellet induced inflammation of EEBR as well as EEPA showed appreciable and dose related efficacy in all the administered doses.

The efficacy of any hepatoprotective drug is dependent essentially on its capability of reducing the harmful effects of hepatotoxin and in maintaining the normal hepatic physiological mechanism. Hepatoprotective activity of EEBR and EEPA were studied in CCl₄ and Paracetamol induced liver injury in rats using N-acetyl-l-cysteine and Silymarin as standard drugs respectively. The curative efficacy of administered extracts was dose dependent as evidenced by gradual reversal of the altered values of various biochemical markers back to normal following oral administration. This may, probably be through promotional activation of antioxidative enzymes and regeneration of hepatocytes that restore the structural and functional integrity of liver. The protective effect observed on treatment with the tested extracts strongly indicates that the extract may have the ability to mitigate any leakages of marker enzymes into circulation, condition the hepatocytes to
hasten regeneration of parenchymal cells, and preserve the integrity of the plasma membranes.

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal places and central veins. Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty erosion were observed in paracetamol and CCl₄ intoxicated animals.

In the Oral glucose tolerance test the tested extracts were found to equally effective with the standard drug Glibenclamide. Further, the tested extracts have shown a dose dependent decrease in the serum glucose levels on 7th, 14th and 21st day on streptozotocinized rats. The plausible mechanism behind the antidiabetic potential of tested extracts could be due to the presence of tannins, flavanoids and phytosterols which would have augmented the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway and also due to extra-pancretic effects or regeneration of β-cells in pancreatic islets.

The present study demonstrated that EEBR & EEPA were capable of promoting wound healing activity. Enhanced wound contraction and histological observations suggest that both plants have potential in the management of wound healing and suggest further study.

The extracts also reduced elevated body temperature in the yeast induced pyrexia model without falling below normal body temperature. All the pharmacological studies suggested that the tested extracts
contain many number of active principles that are responsible for
exhibition of such activities. Hence, the extracts were subjected to
isolation of the individual active principles and its structural elucidation
by subjecting to different spectral procedures.

The IR, $^1$H NMR, $^{13}$C-NMR and MS spectral data suggests that
EEBR$_1$ may be “Stigmasterol”, EEBR$_2$ it may be “β-Sitosterol”, EEPA$_1$
may be “Keampferol” and EEPA$_2$ may be “Quercetin”.

Two sterols were isolated from the ethanolic extract obtained
from the leaves of Basella rubra. The structures of the isolated new
compounds were identified as Stigmasterol (1), and β-sitosterol (2) on the
basis of spectroscopic and by comparing their physical properties
reported in the literature. The complete $^1$H and $^{13}$C NMR spectral
assignments of the two isolated compounds were completed based
on COSY, HSQC, HMBC and MS spectroscopic data.

Finally we isolated and identified the yellow colour crystals of the
kaempferol and quercetin. From the present study it can be confirmed
that Phyllanthus acidus can produces active ingredients Kaempferol and
Quercetin. Although Kaempferol and Quercetin have the potency of a
suitable lead candidate against various ailments, further optimizations
are need for its inclusion in the future drug discovery.
**Conclusion:**

In the light of the above consideration, it can be concluded that the ethanolic extracts of leaves of *Phyllanthus acidus* and *Basella rubra* were found to be safe for internal administration. The extracts were found to possess analgesic, anti-inflammatory, antidiabetic, hepatoprotective, antipyretic and wound healing activities. The IR, $^1$H NMR, $^{13}$C-NMR and MS spectral data suggests that EEBR$_1$ may be “Stigmasterol”, EEBR$_2$ may be “β-Sitosterol”, EEPA$_1$ may be “Keampferol” and EEPA$_2$ may be “Quercetin”.

**Recommendations:**

Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with that of costly synthetic drugs that have adverse effects. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. The present study was conducted to develop newer lead for better and safer therapeutic agents. Further studies are needed to identify the pure component and establish the exact mechanism of actions of the plant extracts. As the plants were safe in animal models they can be explored for clinical trials and can be recommended for using polyherbal formulations.
Additional effort at molecular level should be carried out to establish the exact mechanism of action for all therapeutic actions of the plant extracts and isolated compounds. In comparison with established standard drugs used in the studies, these lead molecules can be taken for further improvement.

Finally, for the development of better therapeutic agent for clinical assessment, detailed pharmacology and toxicology, including genotoxicity and reproductive toxicology studies need to be performed in order to generate data on the potential short and long-term toxicities as well as affirmed pharmacological actions. The discovery and the application of such natural drugs will play a role in human as well as veterinary medicine in the future.