

**ACTIVITY GUIDED ISOLATION OF PHYTOCHEMICAL
CONSTITUENTS OF SOME INDIGENOUS MEDICINAL
PLANTS TO STUDY THEIR EFFECT ON GASTRIC AND
DUODENAL ULCERS**

A THESIS

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7. SUMMARY AND CONCLUSION

The present study was guided with an intention of isolating the phytoconstituents from the leaves of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* based on their activity on different gastric and duodenal ulcer models.

In the current study the leaves of *Raphinus sativus* (Cruciferae), *Moringa oleifera* (Moringacea) and *Amaranthus tricolor* (Amaranthaceae) were gathered from the areas of Bengaluru and Tumkur, Karnataka. Taxonomical identification was done by the Botanist of university of agricultural sciences, Bengaluru. The leaves were dried out under shade and mechanically grinded to coarse pulverize material (#40) to aid for extraction process.

The coarse leaf powder of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* thus formed were extracted by cold maceration technique using various solvents like ethanol (90%), pet-ether (40-60°C), chloroform and ethyl acetate. The leaf fractions of the individual plants with specific solvents thus obtained were concentrated and subjected for phytochemical evaluation.

The phytochemical evaluation results revealed the existence of carbohydrates, proteins, flavonoids, steroids and tannins as the main constituents.

To fix the dose of the extracts for pharmacological evaluation, the different leaf fractions of *Raphinus sativus*, *Moringa oleifera* and

Amaranthus tricolor were tested for acute oral toxicity as per the OECD guidelines #425 (up and down procedure). As no mortality was found at the tested dose (2000mpk), 1/10th of this dose (i.e.200mpk) was fixed for pharmacological evaluation in rats.

The different leaf fractions of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* were preliminarily evaluated for antiulcer activity using two gastric ulcer models in rats (i.e. acetic acid induced chronic model and pylorus ligation induced ulcers in rats). The leaf fractions which exhibited ulcer protective action were further tested in other gastric ulcer models (i.e. ethanol induced, indomethacin induced, ischemia-reperfusion induced gastric ulcers and cysteamine induced duodenal ulcers in rats). The results revealed that the ethanolic leaf extract and ethylacetate leaf fractions of the *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* were effective in all the ulcer models tested, with the ethylacetate fractions stood potent. While the chloroform fraction of *Raphinus sativus* and the petroleum ether fraction of *Moringa oleifera* were found to be effective in healing the acetic induced gastric ulcers. The other leaf fractions were found to be ineffective in healing the ulcers.

Since the ethylacetate fractions of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* were found to have potent antiulcer activity; an attempt was made to isolate the major phytoconstituents existing in them by adopting various chromatographic methods (TLC, CC, HPLC).

This resulted in isolation of two compounds each from the ethylacetate leaf fractions of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor*.

The characterization and structural elucidation of the isolated compounds were done by employing various spectral methods like U.V, FTIR, NMR and Mass spectroscopy. The spectral data obtained were interpreted with the literature data and finally the conclusion was drawn as follows:

- i. The two compounds isolated from the ethylacetate leaf fractions of *Raphinus sativus* were characterized as flavonoids namely **kaempferol** and **kaempferol-3-rahmnoside**.
- ii. The two compounds isolated from the ethylacetate leaf fractions of *Moringa oleifera* were characterized as flavonoids namely **quercetin** and **quercetin-3-rutinoside**.
- iii. The two compounds isolated from the ethylacetate leaf fractions of *Amaranthus tricolor* were characterized as phenolic acids namely **coumaric acid** and **ferulic acid**.

The yields of the phytoconstituents isolated were not sufficient to evaluate *in-vivo* antiulcer activity, hence an effort was drawn to evaluate their H^+-K^+ -ATPase inhibition ability and *in-vitro* antioxidant activity and thereby to correlate the *in-vivo* activity obtained from the crude fractions.

The antioxidant activity was evaluated using DPPH and NO scavenging assay. The compounds isolated from the ethylacetate leaf

fractions of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* were found to have a potent H^+K^+ -ATPase inhibition, potent DPPH and NO scavenging ability which were comparable to that of the positive controls omeprazole and ascorbic acid.

Apart from this, effort was made to formulate a polyherbal formulation having the ethylacetate leaf fractions of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* which was assessed for its antiulcer activity by employing different gastric ulcer models in rats. The results suggested that the PHF was quite effective in healing the ulcers concluding the synergistic effects of the extracts combined.

Based on the overall outcome of the current study we can conclude that the leaves of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* are known to have antioxidant components and the consumption of those leaves may be helpful in peptic ulcer disease patients.

Finally to conclude, the antiulcer activity of the ethylacetate fractions of *Raphinus sativus*, *Moringa oleifera* and *Amaranthus tricolor* might be due to the presence of flavonoids as the major phytoconstituents.
