is malondialdehyde (MDA), one of the secondary lipid peroxidation products. These carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases (27). Thus the decrease in the MDA levels in the presence of increased concentration of each decoction indicates the role of decoctions as antioxidants. TBARS assay was used to determine the antilipid peroxidation properties of the three decoctions. Egg yolk was used as the lipid rich substrate (26, 27). However, minor change to the procedure had to be adopted as the water extracts of all three decoctions have a colour of dark brown to red which interfered with the resultant colour of malonaldehyde - thiobarbituric acid adduct. Hence a blank for each concentration of every drug was prepared which contained, the sample, thiobarbituric acid, acetic acid and butanol. All the investigated decoctions show protective antioxidant activity at different magnitudes of potency. Vitamin E was used as the positive control in the anti lipid peroxidation assay. According to the results obtained, all three decoctions show high anti lipid peroxidation abilities than vitamin E over the concentration range used. There is no significant difference ($p>0.05$) between decoction D1 and decoction D2 in antilipid peroxidation activity. The high antilipid peroxidation activity of decoctions, D1 and D2 may be attributed to their high phenolic contents. Decoction D3 showed significantly low ($p<0.05$) anti lipid peroxidation activity compared to D1 and D2, under the concentrations investigated.

Anti lipid peroxidation activity of some individual constituents of decoction D1, D2 and D3 has been reported. A dose dependent relationship was observed when aqueous acetone extracts of *Terminalia chebula, Terminalia bellerica*, and *Phyllanthus emblica* were tested for their ability to reduce lipid peroxides generated by $Fe2^{+}$ Ascorbic acid system on mice liver homogenate (17). A study carried out by Naik et al. (16) shows that, Triphala and its major constituents inhibit γ-radiation induced damage in microsomal lipids. Lee et al., (20) studied the anti lipid peroxidation ability of methanol extract of *Smilax china* root, which was one of the constituent of decoction D2. This study was conducted on H_2O_2 treated V79-4 cells where the IC_{50} value was found to be >100 mg/ml. Effect of *Nigella sativa* essential oils, (Thymoquinone, carvacrol and 4- terpineol) on lipid peroxidation of bovine brain extract liposomes has also been reported (19, 21). Since they have used different lipid rich substrates and peroxide radical inducing agents such as ascorbic acid, H_2O_2 and Fe^{2+}ions, it is difficult to make a direct comparison between our results and results found in literature. However, they provide supportive evidences to our results on antilipid peroxidation ability of the drugs present in the decoctions investigated.

For the measurement of the reducing ability, the Fe^{3+}-Fe^{3+} transformation was investigated in the presence of water extracts of each decoction. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (32). Similar to their antioxidant activities, the reducing power of each decoction increased with increasing dosage. L Ascorbic acid showed remarkably higher reducing power than the all three decoctions investigated. The decoction D3 showed very low reducing power as well as low antioxidant activity, which can be associated with its low phenol content.

In addition to antioxidant and anti lipid peroxidation activities of decoction D1, D2 and D3, cytotoxicity studies were carried out using LDH and MTT assays. Quantitative analysis of lactate dehydrogenase (LDH) release is based on the fact that LDH is a strictly cytoplasmic enzyme and the elevation of its level in the culture medium reflects the disruption of the host cell plasma membrane (33). Recent studies suggest that LDH is a more reliable and accurate marker of cytotoxicity, because damaged cells are fragmented completely during the course of prolonged incubation with substances (34). The MTT cell proliferation assay measures the cell proliferation rate. This test is based on the conversion of tetrazolium salts into coloured product, formazan, by the mitochondrial enzyme succinate dehydrogenase. Because only metabolically active cells cleave tetrazolium salts, the number of surviving cells is directly proportional to the level of the formazan product created.

The release of LDH was increased in a dose dependent manner after treatment of decoction D1 and D2 over a period of 24 hours, however for Decoction D3, the release of LDH retained at base line level over the concentrations investigated. Parallel results were obtained for MTT assay. In comparison to the results of LDH and MTT assays, it is observed that the results of both assays are in conformity with each other and associate with phenol content of the relevant decoctions. Further LDH and MTT assays reveal that, these decoctions not only inhibited the proliferation of RD cells but also induced cell death.

Agents capable of inducing apoptosis, inhibiting cell proliferation, or modulating signal transduction are

currently used for the treatment of cancer (35). A combination of multiple chemopreventive agents or agents with multiple targets is considered to be more effective than a single agent (35, 36). Kaur and coworkers (37) have identified gallic acid present in Triphala plays an important role in inducing cytotoxicity and apoptosis in cancer cell lines. Deep et al. (38) reported that Triphala inhibited the induction of benzo(a)pyrene induced fore stomach tumoigenesis and such inhibition may be related to the suppression of cell proliferation and the induction of apoptosis. Among individual ingredients of the decoctions investigated, *Terminalia chebula, Phyllanthus emblica, Commiphora mukul*, *Smilax china* and *Nigella sativa, which* present in decoction, D1 and D2 were reported to have anticancer effects on cancer cells (15, 22, 23, 39). Further Khan et al. (40) revealed that main compound responsible for antiproliferative activity of *Phyllanthus emblica* is Phyrogallol. Samudio et al. (22) have reported antileukemic effects of three steroids, cis-gugulstterone, trans-gugulsteron, and 16-dehydroprogesteron, which are some active components present in the gum resin of *commiphora mukul*. Our study shows that there is a link between *in vitro* cytotoxicity and the total phenolic content of the three decoctions D1, D2 and D3. Some constituents such as *Rubia cordifolia, Picrorhiza kurroa, Azadirachta indica,* and *Perocarpus santalinus* in decoction D3 have been studied for their anticancer properties (41-43). However, to our knowledge no study has been done on the synergistic effect of the components in the decoction D3 towards their anticancer and antioxidant activities.

As a conclusion, it can be stated that the results obtained from the present study clearly showed decoction D1 and D2 had strong and effective antioxidant antiproliferative and cytotoxic activities

Table 1: *Extraction yield, Total phenolic content, DPPH radical scavenging activity and anti lipid peroxidatin activity of decoction D1, D2, D3 and Standard compounds.*

Decoction	Extraction yield (mg/g dry matter)	Total phenol content (%w/wgallicacid equivalents)*	DPPHradical scavenging activity EC_{50} (µg/ml)*	Antilipid peroxidation activity. (TBARS assay) EC_{50} (mg/ml)**
Decoction D1	250	37.5 ± 1.4	6.8 ± 0.0	2.2 ± 0.2
Decoction D2	200	30.5 ± 0.7	7.3 ± 0.1	2.2 ± 0.1
Decoction D3	84	6.4 ± 0.3	140.9 ± 1.6	3.0 ±0.1
Ascorbic acid	-	-	6.4 ± 0.1	-
Vitamin E	-	-	-	4.0 ± 0.1

Data represented as the mean ± S.E.M (n=6) ; Data represented as the mean ± S.E.M (n=4); EC_{50} value was defined as the concentration of 50% inhibition of respective radical

Table 2 : Comparison of dose dependent LDH leakage in RD cells after exposure to decoctions D1, D2 and D3 for 24 h. Data are presented as percentage LDH released to that of control ±SEM (n=3).

Decoction	**Concentration . µg/ml)**	**% LDH release**
Decoction D1	0	0
	50	2.4 ± 0.7
	100	72.4 ± 7.4
	150	93.4 ± 0.5
	200	95.3 ± 0.6
Decoction D2	0	0
	50	2.6 ± 0.9
	100	19.2 ± 4.2
	150	68.8 ± 2.3
	200	88.6 ± 4.2

Decoction D3	0	-
	50	-
	100	-
	150	-
	200	-

- No LDH release compared to the negative controls

Table 3. Dose dependent effects of decoctions D1, D2 and D3 on cell viability of RD cell line. Viability was determined by MTT assay following exposure to decoctions for 24 h. Data are presented as mean of percentage viable cells to that of control in three independent experiments.

.Decoction	Concentration (µgml)	% Cell viability± S.E.M.
Decoction D1	0	100.0
	50	90.4 ± 0.1
	100	29.0 ± 0.4
	150	10.3 ± 1.6
	200	3.2 ± 1.0
Decoction D2	0	100.0
	50	90.5 ± 0.3
	100	73.0 ± 0.7
	150	41.5 ± 0.5
	200	10.1 ± 2.4
Decoction D3	0	100.0
	50	88.6 ± 4.1
	100	82.2 ± 4.7
	150	82.1 ± 4.4
	200	78.9 ± 2.8

compared to the decoction D3. Also it can be inferred that above activities were directly correlate to the total amount of phenolics found in each decoction. The additive roles of phytochemicals may contribute significantly to the potent antioxidant activity and the ability to inhibit cancer cell proliferation *in vitro*. Since this is a preliminary study on the anticancer potential of above selected decoctions, further chemical and pharmacological work at molecular level are required to establish the possible correlation among the investigated activities of the above herbal preparations.

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